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GRAM NEGATIVE ANAEROBIC BACILLI: ISOLATION, INCIDENCE, AND CULTURAL CHARACTERISTICS*

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The gram negative anaerobic bacilli are perhaps the least known of all commonly occuring bacteria. In a review covering these organisms, Dack (1940) stated that although they were discovered about the turn of the century, they "are as little known to most bacteriologists today as they were thirty years ago. The texbooks of bacteriology give little if any discussion of them, so that it becomes a major research problem for the laboratory worker to identify any of these species when encountered." Now, twenty years after the appearance of this review, the situation seems to be unchanged.

This lack of attention is not because these organisms are rare. They are normally found in mucous membranes, particularly those lining the lower intestine and the female reproductive tract. They occur in enormous numbers in normal stools (Eggerth and Gagnon, 1933). Pathologically, they may be recovered from local lesions near the sites of their normal habitat or they may at times cause generalized infections such as septicemia or meningitis.

The purpose of this report is to emphasize the frequency of infections with these organisms and to show the ease with which they can be isolated and recognized in the clinical laboratory.

No attempt will be made here to elucidate the taxonomy of this group. The most recent edition of Bergey's Manual (Breed, et al., 1957) places gram negative anaerobic bacilli in the family *Bacteriodaceae*. The family comprises five genera: *Bacteroides, Fusobacterium, Sphaerophorus, Dialister,* and *Streptobacillus.* Members of the genus *Fusobacterium* are characterized by tapering or pointed ends; those of *Dialister* by cell diameters of less than 0.15 micron. *Streptobacilli* are not strict anaerobes.

The organisms reported upon in this paper belongs either to *Bacteroides* or *Sphaerophorus.* For convenience and for other reasons to be discussed later, they will be referred to throughout as *Bacteroides.*

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Methods and Results

Isolation

It was found in this laboratory that several strains of *Bacteroides* were not sensitive to very high concentrations of kanamycin. As this antibiotic is known to be stable to autoclaving and has a wide spectrum of activity against aerobic organisms, it was thought that kanamycin might be useful as a selective agent for the cultivation of *Bacteroides*. All strains were resistant to 750 mcg. per ml., the highest concentration tested. This level of antibiotic is well above that required to inhibit the growth of many other organisms.

Figure I shows the results of an experiment in which five different species of organisms were inoculated into plain thioglycollate medium and into thioglycollate containing 500 mcg. per ml. of kanamycin. It can be seen that *Escherichia coli, Proteus, Pseudomonas, and Clostridium sporogenes* were inhibited at this concentration whereas *Bacteroides* grew equally well in the presence of the antibiotic as in its absence.

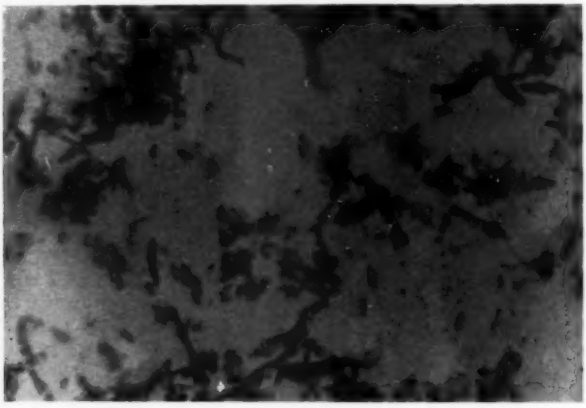

Figure I
Miscroscopic View of Typical *Bacteroides*
Magnification approximately X 1,300.

As a result of these experiments it became routine practice in this laboratory to inoculate all specimens suspected of containing *Bacteroides* into a tube of thioglycollate medium containing kanamycin.

Such a medium is conveniently prepared by adding 1.5 ml. Kantrex solution (Bristol Laboratories, kanamycin sulfate, 1 gm. per 3 ml.) per liter of fluid thioglycollate medium (Difco). The medium is then tubed and autoclaved as usual.

Routine use of this medium facilitated recovery of *Bacteroides* by reducing the competition of other organisms. Due to the presence of some kanamycin-resistant organisms, such as anaerobic streptococci, *Bacteroides* were not always obtained in pure culture; however, even in these cases, competition was sufficiently reduced so that pure cultures could be obtained by further methods.

For this purpose, anaerobic blood agar plates as described by Carlquist (1959) were found to be useful.

Incidence

A review of the records of this laboratory reveals that by the use of the thioglycollate-kanamycin medium, *Bacteroides* were recovered fifty times in a period of fifteen months. Excluding cultures examined solely for acid-fast bacilli, this represents approximately one percent of all cultures examined during this time, and 4.5 per cent of all cultures from local lesions.

A comparison with the results reported by others indicates that this incidence of cultures positive for *Bacteroides* is higher than that usually encountered. This may be seen by examination of Table I which lists total cases, and time covered in some previous studies. In the table the column showing mortality is included to stress the serious nature of some *Bacteroides* infections.

TABLE I

Incidence of Bacteroides Infections Reported By Other Authors

Authors	Total Cases	Time Covered	Mortality
Dack (1940)	105	4 Yrs.	..
Smith and Ropes (1945)	20	48 Mos.	3
Beigelman and Rantz (1949)	47	8 Yrs.	..
Clark and Wiersma (1952)	47	28 Mos.	..
McVay and Sprunt (1952)	35	60 Mos.	11
Carter, et al. (1953)	153	14 Yrs.	13
Fisher and McKusick (1953)	14	72 Mos.	2

Bacteroides infections often arise from sites adjacent to the normal habitat of these organisms. In this series, most isolations were obtained from abdominal abscesses and from the female genital tract. Table II shows the number of isolations by sources of specimens. Also shown is the number of times cultures were obtained pure or mixed with other organisms. Table III lists the various organisms found mixed with *Bacteroides*. It should be pointed out that these other organisms were obtained on routine culture media which were used in addition to the thioglycollate-kanamycin medium.

TABLE II

Origins of Specimens From Which Bacteroides Were Isolated

Sources	Isolations*	CULTURES	
		Mixed	Pure
Abdominal abscesses	13	9	4
Female reproductive system	10	8	2
Perirectal abscesses	6	3	3
Spinal fluid	5	2	3
Ear and mastoid	3	3	..
Appendix	2	2	..
Groin	2	1	1
Leg and hip ulcers	2	2	..
Tongue and lip ulcers	2	2	..
Brain abcess	1	..	1
Miscellaneous	4	3	1

* Figures under this heading refer to isolations, not cases. For example, **Bacteroides** were isolated five times from three cases of meningitis.

TABLE III

Organisms Found Mixed With Bacteroides In Clinical Specimens

Bacteroides		Number of Isolations
Bacteroides in pure culture		11
Bacteroides mixed with other anaerobes		6
Clostridium	2	
Anaerobic Streptococcus	4	
Bacteroides mixed with microaerophiles		13
Lactobacilli	2	
Streptococci	11	
Bacteroides mixed with aerobes (including facultative anaerobes)		22
Staphylococcus	7	
Escherichia coli	7	
Proteus	3	
Pseudomonas	3	
Diphtheroides	2	

Cultural Characteristics

When *Bacteroides* have been obtained in pure culture they may be easily maintained in cooked meat broth or thioglycollate. Cultures in cooked meat medium kept at room temperature have remained viable as long as five months. In thioglycollate medium, however, more frequent subcultures are necessary to maintain viability.

Growth in these media is usually evident in 24-48 hours at 37°C. In thioglycollate or thioglycollate-kanamycin medium growth first appears near the center of the tube with a flocculent appearance. Later, the growth is diffused throughout the tube with a gray-white sediment at the bottom. Gas production is present but is usually not evident until growth is disturbed. The cultures have a definite odor, but this is not the foul, putrefactive odor often encountered with other anaerobes.

Growth is always more abundant in broth than on agar. The addition of five per cent human blood to brain heart infusion agar increases the amount of growth obtained. Yeast extract does not replace the stimulatory effect of whole blood.

Microscopically, *Bacteroides* from broth cultures are found to be nonmotile gram negative bacilli. Non-pleomorphic strains measure approximately 1.5—3.0 microns, but pleomorphic forms may be considerably larger. One of the most striking characteristics of these latter forms is the production of large bodies, which are not heat-resistant, and should not be mistaken for spores. Figure II shows the microscopic morphology of a typical pleomorphic strain.

Pleomorphism is rarely observed on agar. Colonies on blood agar, following 24 hours incubation under anaerobic conditions, are small, gray-white, moist, opaque, and round with entire edges. Although some authors have reported hemolytic strains, none of the strains studied here was hemolytic on human blood agar. Odor is more marked on blood agar plated than in broth. The best way to describe this odor is that of a wet, dirty dog!

Physiological studies were performed on twelve strains selected at random. Action on carbohydrates was tested in tubes of broth over-layed with paraffin and in shake tubes. The medium used was phenol red broth or agar (Difco) to which was added one per cent of the carbohydrates (glucose, sucrose, maltose, lactose, and mannitol).

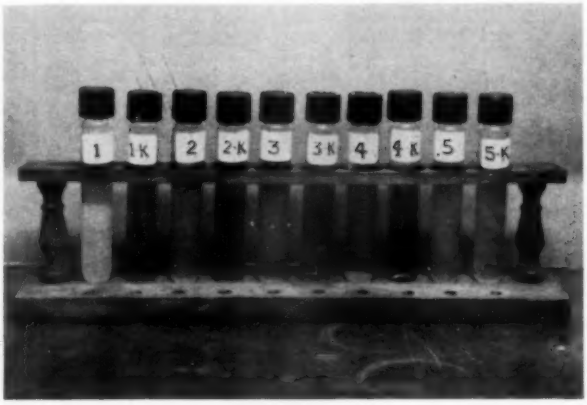

Figure II
Effect of Kanamycin on Various Bacteria
Tubes marked "K" contain 500 mcg. of Kanamycin in thioglycollate medium.
Organisms are: 1. *E. Coli*, 2. *Proteus*, 3. *Pseudomonas*, 4. *Clostridium sporogenes*, and 5. *Bacteroides*.

All strains produced acid without gas from glucose. Only two of twelve strains produced acid from mannitol. Sucrose was fermented by seven, maltose by eight, and lactose by ten of twelve strains at 94 hours. No consistent pattern was observed although five strains fermented all carbohydrates except mannitol. Among these strains no gas from carbohydrates was produced in seven days. Therefore, it appears that the gas observed in the thioglycollate medium is derived from peptone.

The ability to attack gelatin, casein, and lecithin was tested by growing the organisms on tryptose agar to which had been added gelatin, powdered milk, and egg yolk. None of the strains tested hydrolyzed gelatin, split casein, or gave a precipitate on egg yolk agar following 48 hours incubation under anaerobic conditions.

No growth was obtained on Simmons citrate agar. In shake tubes of TSI agar hydrogen sulfide was not visible at seven days. All strains were indole negative.

Sensitivity to antibiotics was determined on anaerobic blood agar plates using commercially prepared discs ("Multidisks"). All strains tested were resistant to bacitracin, streptomycin, kanamycin, polymixin B, and all were sensitive to erythromycin, furadantin, and the tetracyclines. Table IV shows the results of these tests against twelve strains.

TABLE IV

Antibiotic Sensitivity of Bacteroides

Strains	A	B	C	NV	P	S	T	OL	G	K	PB	SSS	TE	E	F
1	S	R	R	R	R	R	S	S	S	R	R	S	S	S	S
2	S	R	R	S	R	R	S	S	S	R	R	R	S	S	S
3	S	R	R	R	R	R	S	R	S	R	R	S	S	S	S
4	S	R	R	R	R	R	S	R	R	R	R	R	S	S	S
5	S	R	R	R	R	R	S	R	S	R	R	S	S	S	S
6	S	R	R	R	R	R	S	R	S	R	R	S	S	S	S
7	S	R	R	R	R	R	S	R	S	R	R	R	S	S	S
8	S	R	S	R	R	R	S	S	S	R	R	S	S	S	S
9	S	R	S	R	R	R	S	R	S	R	R	S	S	S	S
10	S	R	R	R	R	R	S	R	S	R	R	R	S	S	S
11	S	R	R	R	R	R	S	R	R	R	R	R	S	S	S
12	S	R	S	R	S	R	S	R	S	R	R	S	S	S	S

A——Chlortetracycline, 5 mcg.
B——Bacitracin, 2 units.
C——Chloramphenicol, 5 mcg.
NV——Novobiocin, 5 mcg.
P——Penicillin, 2 units.
S——Streptomycin, 2 mcg.
T——Oxytetracycline, 5 mcg.
E——Erythromycin, 2 mcg.
OL——Oleandomycin, 2 mcg.
F——Furadantin, 50 mcg.
G——Gantrisin, 50 mcg.
K——Kanamycin, 5 mcg.
N——Neomycin, 5 mcg.
PB——Polymyxin B, 50 mcg.
TE——Tetracycline, 5 mcg.
SSS——Triple Sulfa, 50 mcg.
In the body of the chart: S——Sensitivity or no growth of organism.
R——Resistance or growth of organism.

Discussion

Problems of nomenclature present a barrier to better understanding of the gram negative anaerobic bacilli. The organisms reported upon in this paper have been referred to as *Bacteroides*, partly because this is the name which appears to be most firmly entrenched in the medical literature and hence is most familiar to clinicians. Most workers appear to be content with the convention of Smith and Ropes (1945) of designating pleomorphic strains as *Bacteroides funduliformis* and non-pleomorphic strains as *Bacteroides fragilis*. It is on this basis, however, that the Bergey classification separates the genera *Bacteroides* (non-pleomorphic) and *Sphaerophorus* (pleomorphic).

All of the strains reported upon here were pleomorphic on primary isolation. It was observed that in subcultures pleomorphism was often less marked although some pleomorphic cells, including balloon shaped forms, could usually be found. Furthermore, the composition of the medium appeared to influence this characteristic. The organisms were always more pleomorphic in broth than on agar, and usually more pleomorphic in thioglycollate than in cooked meat medium.

The physiological tests described here did not facilitate identification of *Bacteroides*. All cultures failed to grow aerobically and were composed of gram negative rods, pleomorphic on primary isolation. All produced acid without gas from glucose, were indole negative, and on solid media, grew better in the presence of blood. These characteristics indicated that these cultures belonged to a single large group, with some variation in fermentative abilities among strains.

Three of the cultures in this series came from cases of meningitis, one of which was fatal. This emphasized the fact that these organisms can cause serious infections and should be looked for from all sources. Antibiotic sensitivity tests indicate that the organisms are not particularly resistant, and in a number of instances at this hospital, prompt clinical response to specific therapy confirmed laboratory sensitivity reports.

Summary

Fluid thioglycollate medium containing the antibiotic kanamycin has been found to be a good medium for isolating gram negative anaerobic bacilli. Routine use of this medium resulted in 50 isolations over a 15 month period. Some cultural characteristics of a dozen strains have been studied and are described.

The importance of looking for these organisms in clinical specimens is stressed.

Acknowledgment

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A CONTROL METHOD FOR ASSURED DAILY ACCURACY OF CLINICAL CHEMISTRY†

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The need for day to day accuracy is perhaps the most important single problem encountered by the medical technologist. He *must* know if his test is within the limits of unavoidable error, and he must also know how to find and correct any existing error in the procedure and technique.

Most laboratories do not have, and cannot afford, the elaborate equipment used in industrial quality control. It is therefore necessary that some means of control and correction be devised using the existing facilities. It is also necessary that the procedure not be too cumbersome or time consuming for the existing personnel to handle. One of the causes of laboratory error is due to too much work load in relation to personnel.[2,3] To overload the technologist with long involved procedures of quality control would be to defeat the purpose of the entire project.

Perhaps the most significant fact in any workable quality control procedure is the degree of confidence it gives the technologist. The physician *must* depend upon the laboratory for accurate results. Many diagnoses are made on the basis of laboratory analysis. The accuracy of these analyses cannot be over-emphasized. *No* laboratory report is better than an inaccurate report.

Correct calibration of all phases of the clinical chemistry procedure is a necessity that cannot be overlooked. All pipettes and glassware should be calibrated when new.[1] Pipettes with broken tips are of no value whatsoever. Cuvettes should be calibrated when new, and at frequent intervals to avoid errors caused by scratches. All procedure calibration should be done with a pure standard. The College of American Pathologists Standards are excellent for this.

We felt that a satisfactory method of control must provide us with the following:

1. An easily read chart or graph of results which could be interpreted by by the chemist or pathologist.
2. Accurate records for future long range studies.
3. A control which could be easily stored and which would be completely reliable.

In addition, we were looking for a method which, with a minimum of extra work, would give us a high degree of accuracy; a method which could be used either in a large or small laboratory; a method that would show (1) if error is present, (2) the amount of error, (3) where the error might be found and (4) correction of the error itself.

Materials and Equipment

The necessary materials and equipment are simple. They are:

1. *A "known value" commercial control*

We use the commercial products such as "Lab-trol"*, "Versatol"**, "Hyland"***, etc., as the basis of our control. Some authors have used pooled

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*Dade Reagents, Inc.
**Warner-Chilcott Laboratory Supply Division
***Hyland Laboratories.

sera as their means of control.[2,3] We feel that these commercial controls have certain advantages. They are easily stored and require no special equipment. Their values are known, and stable, eliminating the probability of error introduced through the control itself. They are more convenient, and do not require costly preparation time. The same analyzed values may be retained for a longer period of time if the products are bought in large quantities of the same lot number.

2. *Two spectrophotometers*

We feel that two instruments are justified in the clinical chemistry laboratory. All of our controls are read on a Bausch and Lomb "Spectronic 20," and a Coleman "Universal" spectrophotometer. Both, are calibrated for each procedure. Each instrument is, then, an immediate check on the other.

3. *Data recording*

Determined control values are plotted on a graph. A ledger is used to record all transmittance readings, control values, new reagent introduction, bulb changes, etc.

Method of Control

The method of Henry and Segalove[5] is used to determine our 95 per cent confidence limits or unavoidable variation that occurs when any series of analyses is done. Ninety-five per cent confidence limits may be defined as the limits in which nineteen out of twenty observations will fall.[4] The formula is:

$$o' = \sqrt{\frac{\Sigma\ (\bar{x} - x)^2}{N - 1}}$$

in which N = number of determinations
$\bar{x}$ = arithmetic mean of N determinations
Σ = arithmetic sum of N determinations
o' = standard deviation
x = single determination
$(\bar{x} - x)$ = the deviation of a determination from the mean.

To use the formula:

1. Do twenty or more control determinations
2. Determine mean eg. (41% T. = 107 mg. % glucose).
3. Subtract each observation from the mean.
4. Square each difference (d^2).
5. Add squares.
6. Divide sum of squares by $N - 1$. ($N - 1$ = number of determinations minus one).
7. Take the square root of result and this is one standard deviation.

Two standard deviations are allowed; therefore, our final result is multiplied by two. Once the ninety-five per cent confidence limits are established a graph may be drawn showing these confidence limits. (See figure # 1). For example: twenty glucose determinations are done on a commercial control and the confidence limits are established. The manufacturer's value of the control is 107 mg. per cent. Our average per

cent transmittance reading is 41, which is equivalent to 107 mg. per cent glucose. Our ninety-five per cent confidence limits are 9 mg. per cent. To stay within these limits our controls should all read from 98 to 116 mg. per cent glucose. If these observations stray too far beyond the limits something is obviously wrong and in need of correction.

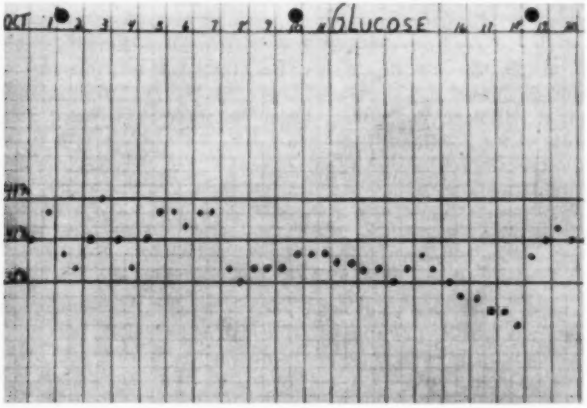

Fig. # 1. A graph illustrating an example of quality control. 41% transmittance = 107 mg. % glucose, 44% transmittance = 98 mg. % glucose, 38% transmittance = 116 mg. % glucose. Control procedures from Oct. 16th through 18th are obviously beyond the 95% confidence limits.

In the application of the method we run a control with the first request each morning. For example, the first glucose ordered is run with a glucose control; the first urea nitrogen is run with a urea nitrogen control. All controls are read on both the Coleman and Bausch and Lomb spectrophotometers. All values are noted on the ledger and each value is plotted on the graph. Unless otherwise indicated, we run only one control a day for each procedure.

If a given procedure goes out of control, our next problem is to find the *source* of the error.

Most errors in clinical chemistry fall into one of four areas:

1. Instrument error
2. Error caused by reagents
3. Error in the control
4. Error in technique.

Some actual examples will probably be the best method to show how these errors are realized, pin-pointed, and corrected.

Results

1. *Error in instrument*

On May 5th, three unrelated procedures; glucose, urea nitrogen and cholesterol all went beyond our limits of control. The cholesterol was off 34 mg. per cent, the glucose 17 mg. per cent and the urea nitrogen

7.0 mg. per cent. All of these readings were made on one instrument. When these same procedures were read on the other instrument, they were found to be within the limits of control. The obvious conclusion, when three unrelated procedures go astray at the same time, is instrument error. A check of our daily ledger showed that an instrument bulb had been changed late the previous afternoon. The bulb was replaced and the procedures read within limits.

2. *Error in reagent*

A urea nitrogen was ordered and run along with a control. The control did not read within the 95 per cent confidence limits on either of our spectrophotometers. This eliminated the probability of instrument error. The only change in procedure was the addition of a freshly prepared reagent. This was replaced and the procedure was back in control.

We have found the best method of check on old reagents is simply looking at the bottles and seeing which ones are the most nearly depleted. In every instance it has been the nearly empty bottle that caused the trouble.

3. *Error in control*

As an example, the chemist noted that the glucose procedure had gone completely out of our predetermined confidence limits. The per cent transmittance had changed from a mean of 40 to 56. The control was then read on our other instrument and the same approximate error was noted. This eliminated the probability of a poor lamp or some other instrument difficulty. A check on the daily ledger showed that no new reagents had been placed in the test. The reagent bottles were all at least half full, eliminating the probability of error due to old reagents. This left only two probabilities, (1) error in technique or (2) error in control. The procedure was done again using the old and a new control. Protein free filtrate was treated with both old and new reagents. This was done to double check the possibility of error introduced by reagents. Results showed the new control to be accurate with both old and new reagents. The old control was still off with both sets of reagents. This is the only error that we have found in our controls. The error was caused by improper storage.

4. *Error due to technique*

When the above checks are made and no cause for error is found, the trouble is usually due to technique. This could be caused by using the wrong pipette, misreading the instrument, dirty glassware, etc. Technical errors are most frequently corrected by repeating the procedure.

Conclusion

The preceding method has been used in our laboratory for the past year and a half. We have found that:

(1) The method is not time consuming, and may easily be run along with the routine daily analyses.

(2) Commercial controls will eliminate one potential source of error.

(3) Errors have been found immediately that otherwise might have gone undetected for some time.

(4) The entire procedure is checked from start to finish. Any error in pipetting, reagents, instrumentation, or technique will show.

(5) Final degree of accuracy may be shown by plotting control values on a graph.

Summary

Ninety-five per cent confidence limits were determined as described by Henry and Segalove.[5] A method has been presented for correcting these procedures shown to be out of control.

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THE USE OF THE SMEAR IN SEX DETERMINATIONS*

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One of the more recent aids in the determination of sex is the method of determining the nuclear sex chromatin pattern.[1] The early work of Barr and his associates[4, 25] in developing methods for the determination of the nuclear sex pattern has opened up a wide field of investigation. They identified a mass of chromatin which could be found in the nuclei of most cells in the female, but which was absent in the male. This sex chromatin mass measures 0.7 x 1.2 microns in mean dimensions, having a rounded, planoconvex, or angular appearance and is mostly found on the inner surface of the nuclear membrane. This mass can be identified only in intermitotic or resting nuclei when the chromatin is generally in a finely granular state. This mass is thought to represent the XX chromosomes of the female.

The genetic sex of an individual is determined at conception by the chromosomal makeup of the spermatozoon effecting fertilization. During the maturation of the germ cells, the number of chromosomes is reduced from 48 to 24, one of each pair going to each daughter cell. Thus, all mature ova contain 23 somatic chromosomes plus an X chromosome, whereas half of the spermatozoa contain an X chromosome and half a Y chromosome. Fertilization of an ovum by an X bearing sperm results in a female, while fertilization by a Y bearing sperm results in a male. This, then is a picture of the genetic sex as is determined by the presence or absence of the sex chromatin mass. However, sex in the early embryo is only potential since the gonad is an undifferentiated cell mass along the urogenital ridge until the sixth to eighth week. Injury to this cell mass and endocrine disturbances may affect development and lead to various types of intersex states or pseudohermaphroditism. There is also some evidence that there are somatic genes for maleness and femaleness affecting all of the body tissues, independent of the genetic sex pattern, and their influence may thus produce a pseudohermaphrodite.[20]

The sex chromatin pattern then is only one of the factors which determines an individual's sex and thus is not necessarily the final sex of an individual.[2, 18, 28] Therefore, it can only be used as an aid to a final determination. The great value of the sex chromatin determination has been in helping to make an early reasonably certain diagnosis of a condition without surgical exploration. Its use is of great importance in the newborn[7] and young children,[33] in conjunction with other tests when sex is in doubt. Needless exploratory surgery is eliminated, future parent-patient anguish and confusion are avoided, and the future needs of the patient anticipated.

The test as originally devised used skin biopsy sections[2, 25] to demonstrate the presence or absence of the sex chromatin mass. This has given way to simpler and more rapid techniques by using epithelial cells removed from oral and vaginal mucosal surfaces. Amniotic fluid has been

* First Award in Histology, Scientific Product Foundations Award, June 1960. Read before the 28th annual convention of ASMT, Atlantic City, New Jersey, June 1960. Received for publication January 1960.

used to determine the sex of the fetus in utero, but this is more for academic interest than a routine procedure. Blood smears which show a different characteristic in the neutrophile leukocytes are also used.

The oral mucosal smear method was introduced simultaneously by Moore and Barr[24] and Marberger et al,[23] and their results have been confirmed by many other investigators.[13,14,16] The smears are prepared by scraping the inner surface of the cheek with a small metal spatula or the edge of a wooden tongue depresser, transfering the material to an albuminized slide and immersing the slide immediately while still moist into a fixing solution of 95% ethyl alcohol or Papanicolaou fixative. The scraping must be brisk enough to obtain more than the surface epithelium, the smear should be thin to avoid overlapping of nuclei and drying before fixation must be avoided. This method is simple and reliable and appears to be the method of choice for routine use.

Carpentier et al[8,9] have observed that vaginal smears taken during routine examinations for cytologic studies and stained by the Papanicolaou technique, clearly demonstrate the sex chromatin body in suitable cells.

The determination of the genetic sex of the fetus in utero by the use of amniotic fluid was first reported by Sacks et al,[29,30] and has been confirmed by other investigators.[12,19,22,31] Amniotic fluid is aspirated directly from the amniotic sac with a needle and syringe. A transabdominal or transvaginal needlepuncture of fetal membranes when done with reasonable care is not dangerous to the mother or child. The desquamated cells are thought to belong to the vagina, oral cavity, or some part of the urinary tract. The fluid is centrifuged and the sediment is spread on an albuminized slide. The slide is immediately immersed into a fixing solution of 95% alcohol or Papanicolaou fixative while still moist. From the end of the fourth month to the beginning of the seventh month of pregnancy, it is a relatively safe and reliable method of diagnosing the sex of the fetus in utero.

Many staining techniques have been used to demonstrate the sex chromatin mass. Some of these methods are simple, but require an expert to interpret the results. Others are quite involved and require expert technique. Barr and his associates used cresylecht violet, the Feulgen reaction, methyl green-pyronin, and hematoxylin and eosin. The Papanicolaou method was used by Carpentier et al. A simplified staining technique using pinacyanole was proposed by Greenblatt.[15] Klinger[21] tried the various staining methods for a critical appraisal and proposed a new method using the acid hydrolysis of the Feulgen reaction and staining with a buffered solution of thionin. The sex chromatin stains deep blue-violet and is sharply contrasted against the lighter colored chromatin of the nucleus. However, all of the above methods do not give a truly differential staining in that the sex chromatin mass is not stained in a contrasting color from the rest of the chromatin. Recently, Guard[17] described two such differential methods. One method used biebrich scarlet and fast green FCF and the other used hematoxylin, biebrich scarlet and fast green FCF. In these two methods there is a competition between two amphoteric dyes and it reveals a selective

retention of biebrich scarlet which stains the sex chromatin red, contrasted with the rest of the chromatin stained by the fast green.

Sex differences by use of the blood smear were introduced by Davidson and Smith,[11] and the method has been used by many others.[5, 6, 10, 26, 32] In routine blood films, they observed a sex difference in the polymorphonuclear neutrophile leukocytes. Those of the female contain an accessory nuclear lobule ("drumstick") attached to a lobe of the nucleus by a thin strand. Six neutrophils or more showing these lobules per 500 cells were an indication of the female sex. The absence of the lobules in 500 cells indicated the male sex. Many minor lobes, sessile nodules, small clubs and tags must not be mistaken for the typical "drumsticks." The "drumsticks" measure 1.5 microns in length with the other appendages being shorter. Routine peripheral blood films are made and stained with Wright's stain. The material is easy to obtain, but the method of observation is tedious and requires an experienced observer. Recently a method for the enrichment of blood smears[27] has been proposed to make the determination easier.

Summary

The use of the nuclear sex determination has become a valuable clinical procedure for the study and planning of the rehabilitation of the sexually disabled so that the clinician can analyze the defects accurately and prescribe an orderly course of treatment. The use of the skin biopsy for this determination has given way to simpler methods by using smears from the oral and vaginal mucosa. Sex differences by the use of neutrophile leukocytes are also noted. The cells in amniotic fluid can be used to determine the genetic sex of the fetus in utero, but this is more for academic interest than a routine procedure. Briefly, some staining techniques are mentioned with Klinger's thionin method and Guard's differential biebrich scarlet-fast green methods propounded to show best the sex chromatin mass.

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BYLAWS

AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

Revised 1953
Amended 1954-1960

BYLAWS
OF THE
AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

ARTICLE I
Name

The name of this corporation is American Society of Medical Technologists.

ARTICLE II
Purposes

The purposes of this Society are to promote higher standards in clinical laboratory methods and research; to elevate the status of those specializing in medical laboratory technique; to create mutual understanding between the medical technologist and physicians and all others who are employed in the interest of individual or public health; to issue charters to constituent societies; to promote the mutual aid and benefits of its members; in general to do anything and everything necessary and proper to the conduct of a society of this nature, and for the purpose of attaining or furthering any of its objects to do any and all other acts and things, and to exercise any and all other powers which now or hereafter may be authorized by law.

ARTICLE III
Constituent and Branch Societies

Section 1. This Society shall be composed of constituent societies and of such branch societies as shall be organized and sponsored by the constituent societies.

Section 2. There may be one, and only one, constituent society in each geographically defined state, District of Columbia, territory, possession or dependency of the United States and in each foreign nation. Any number of branch societies may be organized by a constituent society within the boundary of its geographical area.

Section 3. The name of each constituent society shall contain the geographical location thereof and the words "medical technologists," or the equivalent of these words. A constituent society may use the name of division of the American Society of Medical Technologists.

Section 4. A constituent society can be organized by three or more persons eligible for active membership in this Society. These persons shall petition for permission to organize a constituent society on a form supplied by the executive secretary which shall be submitted to the chairman of the Board of Directors and be approved by that Board. The petition shall be accompanied by an application fee of $5.00. When the petition has been approved, six copies of their proposed constitution and bylaws or, if the group wishes to become incorporated, six copies of their proposed articles of incorporation and bylaws shall be submitted to the constitution and bylaws committee of this Society accompanied by a list of officers and charter members. The committee shall bring a report and a recommendation concerning the chartering of this group to the House of Delegates at the next Annual Session of the Society.

Section 5. A charter for the organization of a constituent society may be granted only by the House of Delegates.

Section 6. The duties and functions of a constituent society are defined as follows:

(a) It shall send to the executive secretary of this Society the names and addresses of its officers not more than two weeks after their election, and the names and addresses of its committee chairmen not more than two weeks after their appointment.

(b) It shall send dues to the executive secretary within two weeks after members have paid, together with a duplicate, typewritten, classified list of such members with their addresses and registry numbers or degree statuses.

(c) It shall provide for the types and qualifications for membership except that it may grant active membership only to persons who meet the requirements for active membership in this Society, that only active members shall have the privilege of voting and holding office in the society; and all members of a constituent society shall abide by the Code of Ethics of this Society.

(d) It shall sustain its status with this Society by maintaining a constitution and bylaws in harmony with the Bylaws of this Society and by maintaining adequate supervision over the branch societies which have been organized under its direction to insure that their constitutions and bylaws are kept in harmony with the Bylaws of this Society. The types of membership and qualifications for membership in a branch society shall be determined by the constituent society under which it is organized, except that all members of a branch society must abide by the Code of Ethics of this Society.

(e) It shall submit to the chairman of the Constitution & Bylaws Committee of this Society twelve copies of all proposed amendments to the constitution and bylaws of the constituent society at least sixty days before the approval of such committee is desired. These amendments should be submitted in context. When the proposed amendments have been approved they may be adopted. A constituent society shall report the adoption of any amendments to its constitution and bylaws to the chairman of the Constitution & Bylaws Committee and must submit a copy of its amended constitution and bylaws for filing in the executive office of this Society within two weeks after their adoption.

(f) It shall send annually before September 15th to the executive secretary a charter fee of $1.00 which provides for the renewal of its charter.

Section 7. This Society shall be empowered to revoke the charter of a constituent society by a two-thirds majority vote of the House of Delegates provided the Board of Directors by a two-thirds vote favors the revocation of such charter and provided, further, that such constituent society shall have had at least thirty days notice of the charges filed against it and shall have had an opportunity to be heard and to defend itself before the House of Delegates. The charter of a constituent society may be revoked for any of the following causes:

(a) Failure of such society to require that all its members abide by the Code of Ethics of this Society.

(b) Adoption by the constituent society of policies or procedures contrary to the purposes of this Society.

(c) Failure of the constituent society to cooperate reasonably with this Society.

Section 8. Any constituent society whose charter has been revoked may be reinstated by a two-thirds vote of the House of Delegates upon application, provided such society is no longer in default and pays all expenses incurred, plus a reinstatement fee of five dollars ($5.00).

Section 9. Any constituent society shall revoke the charter of a branch society organized under its direction for failure of the branch society to comply with both the Code of Ethics of this Society and the bylaws under which the branch society was organized and amendments thereto.

ARTICLE IV
Membership

Section 1. The membership of this Society shall consist of the following classes:

(a) Active Member. Any person who (1) is registered M.T. (ASCP) by the Registry of Medical Technologists of the American Society of Clinical Pathologists and is in good standing with that Registry, or (2) possesses a master's or higher degree in microbiology, biochemistry, hematology, histology, parasitology, or serology from an accredited college, or (3) holds a certificate in chemistry or microbiology from the Registry of Medical Technologists of the American Society of Clinical Pathologists, and is in good standing with that Registry, or (4) holds a limited certificate in histologic technique from the above mentioned Registry and has ninety or more

semester credit hours from an accredited college, of which at least twenty-seven hours are in science including chemistry and/or biology, with lecture and laboratory in each, shall be eligible for active membership in this Society provided, further, that he is an active member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An active member shall have all the rights and privileges of this Society.

(b) Affiliate Member. Any person who holds a certificate from and is in good standing with the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists without meeting the requirements for active membership in this Society shall be eligible for affiliate membership in this Society provided that he is a member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An affiliate member is entitled to all the privileges of membership in this Society with the exception of voting, holding office, and serving on any Board or standing or special committee of this Society.

(c) Student Member. Any person enrolled in an approved School of Medical Technology or in an undergraduate program which is pre-requisite to enrollment in an approved school is eligible for student membership in this Society. He may enjoy this membership until he becomes a registered M. T. (ASCP) by the Board of Registry of Medical Technologists of ASCP or until he becomes ineligible to take the examination for such registration. Upon successful completion of the Registry examination he shall automatically be billed for active membership in this Society. A student member is entitled to all the privileges of membership in this Society except voting, holding office, and serving on any Board or standing or special committee of this Society.

(d) Honorary Member. Any person who has made an outstanding contribution to the field of laboratory medicine by his research or by his service to the profession of medical technology may be recommended for honorary membership in this Society. Recommendations for honorary membership may be made by a constituent society or by the Board of Directors. A recommendation accompanied by qualifications shall be sent to the recording secretary of this Society not less than thirty days before the Annual Session. Honorary members shall be elected by a two-thirds vote of the House of Delegates. They shall be elected every third year beginning in 1954. The year when honorary members are elected, each constituent society and also the Board of Directors shall be entitled to present the name of only one candidate for this honor. Honorary members shall not exceed in number one per cent of the active members of the Society in good standing at the time of their election to honorary membership. An honorary member shall be informed of his election by the recording secretary who shall send him a certificate of honorary membership signed by the president and the recording secretary. He shall enjoy all the privileges of membership except voting, holding office and serving on any Board or standing or special committee of this Society. He shall be exempt from payment of annual dues.

(e) Corresponding Member. Any person who is resident of, and employed as a medical technologist in, a foreign country, who is in good ethical standing, and who is a member of a government-and/or medical association-recognized association of medical technologists in that country, shall be eligible for corresponding membership in this Society. He shall make application through the association of which he is a member. A corresponding member shall enjoy all the privileges of this Society except voting, holding office, serving on any Board or standing or special committee of this Society or any of its activities from which he might be excluded by laws governing aliens. A corresponding member shall forfeit all privileges of membership upon changing his place of residence or employment to a geographical area where there is a constituent society of this Society.

Section 2. Application for active or affiliate membership in this Society shall be made on a form prescribed by this Society. The transmittal to the executive secretary of a list of active and affiliate members in good standing in a constituent society, together with their annual dues shall establish them as members of this Society.

Section 3. Application for student membership in this Society shall be made on a form prescribed by this Society. The transmittal of this application to the executive secretary of this Society together with the required amount of dues shall establish him as a student member of this Society.

Section 4. Any member of this Society whose conduct is detrimental to this Society may be expelled by a two-thirds vote of the House of Delegates provided he has been given thirty days notice, signed by a majority of the members of the Board of Directors and sent by registered mail, stating the charges filed against him and informing him of the date of the meeting when his expulsion will be voted upon. The member so charged shall have the privilege of appearing before the House of Delegates and he may present his defense in person or through other active members of the Society of his selection.

Section 5. Any person who has been expelled from membership may be reinstated upon adoption by a two-thirds vote of the House of Delegates of his application for membership, provided the same is accompanied by annual dues to this Society plus a reinstatement fee of five dollars ($5.00).

ARTICLE V

Code of Ethics

Being fully cognizant of my responsibilities in the practice of Medical Technology, I affirm my willingness to discharge my duties with accuracy, thoughtfulness, and care.

Realizing that the knowledge obtained concerning patients in the course of my work must be treated as confidential I hold inviolate the confidence (trust) placed in me by patient and physicians.

Recognizing that my integrity and that of my profession must be pledged to the absolute reliability of my work, I will conduct myself at all times in a manner appropriate to the dignity of my profession.

ARTICLE VI

Meetings

Section 1. The Society shall hold an Annual Session which shall include one or more general or scientific meetings of the members and not less than two meetings of the House of Delegates, at least two of which shall be held on different days. The Annual Session shall be held at the time and place determined by the House of Delegates at any preceding Annual Session. Emergency changes concerning the time and place of an Annual Session may be made by the Board of Directors.

Section 2. The Board of Directors shall meet within the week following the sine die adjournment of the House of Delegates to transact necessary business and shall hold a meeting within the two weeks prior to the first meeting of the House of Delegates at each Annual Session, and may, if necessary, have an interim meeting between the post and pre-convention meetings to be called by the president.

Section 3. The Advisory Council shall meet within the week following the sine die adjournment of the House of Delegates to transact necessary business and shall hold a meeting within two weeks prior to the first meeting of the House of Delegates of each Annual Session.

ARTICLE VII

Finances

Section 1. The dues for active and affiliate membership in this Society shall be twelve dollars ($12.00) per annum.

Section 2. (a) Dues for student membership in this Society shall be $3.00 per annum. (b) Any dues paid by a student member who becomes eligible and applies for active membership prior to January 1 will be refunded on the basis of $.25 per month for any overlapping period retroactive to July 1.

Section 3. The dues for corresponding membership in this society shall be four dollars ($4.00) per annum.

Section 4. (a) Dues to this Society shall be payable to the treasurer of the constituent Society of the geographical area in which the member is

employed, resides, or maintains his status as a student, or in the event no such constituent society exists, to the executive secretary of this Society.
(b) Annual dues for active, affiliate and corresponding members to this Society are payable on or before July 1 of each fiscal year.
(c) Dues for a student member may be renewed at the end of 12 months for another year, provided he has not in the interim become eligible for active membership in this Society.

Section 5. (a) Any active, affiliate, or corresponding member who cannot pay dues for the current fiscal year shall notify this Society by sending written notification direct to the executive secretary not later than September 1st of that year.
(b) Any active, affiliate, or corresponding member who fails to pay annual dues to this Society by September 1st shall have his name removed from the membership list.
(c) A person whose name has been removed from the membership list of this Society may be reinstated at any time provided he meets the requirements for membership of both his constituent society and this Society. Unless a person has previously resigned, to be reinstated in this Society he shall pay current annual dues to his constituent society with any back dues or reinstatement fee the constituent society may require, and current year's dues to this Society plus a reinstatement fee of two dollars to this Society.
(d) Any active or affiliate member who changes his residence or place of employment to another state, territory or nation shall become a member of the constituent society of his new residence or place of employment for the remainder of the fiscal year without further payment of dues.

Section 6. A new applicant elected to active, affiliate, or corresponding membership in this Society after January 1st and prior to May 1st shall pay one-half of the annual dues to this Society for the remainder of the fiscal year.

Section 7. An applicant elected to active, affiliate, or corresponding membership in this Society between May 1st and June 30th shall pay annual dues for one year which shall entitle him to all the privileges of his respective membership for a period up to fourteen months. He shall pay no further dues to this Society until the second fiscal year following the date of his application.

ARTICLE VIII
Officers

Section 1. The officers of this Society shall be president, president-elect, recording secretary and treasurer.

(a) The president shall be the chief executive of this Society and shall preside at all meetings of the Board of Directors and of the House of Delegates. He shall be an ex officio member of all standing and special committees except the nominations committee. He shall set up such standing committees as are provided for in the Bylaws except the nominations committee. He shall appoint with the approval of the Board of Directors, such special committees as are needed.

(b) The president-elect shall, during his term of office, familiarize himself with the duties which shall devolve upon him as president. In the event of the death, resignation or total disability of the president, the president-elect shall perform the duties of, and have the same authority as, the president.

(c) (1) The recording secretary shall act as secretary at all meetings of the Board of Directors and of the House of Delegates.

(2) He shall keep in permanent form in his possession a record of all minutes taken at all meetings at which he serves, and shall provide each of the members of the Board of Directors and the executive secretary with a copy of these minutes within thirty days after the close of the Annual Session at which the minutes were taken.

(3) He shall, assisted by the minutes committee, prepare an abstract of the minutes to be published and distributed to the membership.

(4) He shall carry on such correspondence as shall be authorized by the House of Delegates and Board of Directors of this Society.

(5) To aid the recording secretary in the keeping of an accurate record of the annual meetings of the House of Delegates the Society shall employ a mechanical recording device or an expert stenographer to record all the proceedings of the meetings of the House of Delegates. Such record shall be delivered to the president of this Society by the stenographer within sixty days after the close of an Annual Session. The president shall within sixty days after receipt of the record transmit the same to the executive secretary to be filed in the executive office.

(6) In the event of the simultaneous inability of both the president and the president-elect to perform the duties of president, the recording secretary shall perform the duties of, and have the same authority as, the president.

(d) The treasurer shall receive, record and have charge of all funds of this Society. He shall deposit these funds in a bank designated by the Board of Directors and pay all bills approved by the Board of Directors or the House of Delegates upon receipt of vouchers signed by the chairman of the Board. He shall be bonded at the expense of the Society. He shall, within sixty days previous to the Annual Session, have his accounts audited by a public accountant at the expense of the Society. He shall submit to the House of Delegates at each Annual Session an itemized statement of all receipts and expenditures of the Society for the year just ended, and a copy of this itemized statement shall be distributed to each delegate prior to the reading of the report. At the end of his term of office he shall have a final interim audit of his accounts and shall deliver his current records and all cash on hand to his successor. All other records in his possession shall be transmitted to the executive secretary to be filed in the executive office. He shall serve as an EX OFFICIO member of the Finance Committee during his term of office, disregarding the amount of time he may have served as an appointed member of that committee prior to his election to office.

(e) At the end of the term of office by expiration or otherwise each officer, except the treasurer, each Board member and Council member, shall deliver his official records and correspondence to his successor for transmittal to the executive secretary to be filed in the executive office.

Section 2. Eligibility for Office. Any person who is, and for at least three years prior to his election or appointment has been, an active member of this Society shall be eligible to hold office.

Section 3. Method of Election.

(a) All officers of this Society shall be elected by the House of Delegates at the Annual Session of this Society.

(b) An official list of nominees for each office shall be presented to the House of Delegates by the nominations committee, the list of candidates and their qualifications having been sent to each member of this Society at least sixty days before the Annual Session. Nominations may be made from the floor. The qualifications of all persons nominated from the floor shall be presented to the House of Delegates at the time such nominations are made.

(c) The election of officers by the House of Delegates shall be conducted by an elections committee consisting of five members of the House of Delegates appointed by the chair.

(d) Election shall be by ballot and a majority of votes cast elects. If none of the candidates for a certain office receives a majority vote on the first ballot, the candidate with the lowest number shall be eliminated and balloting shall continue until a candidate receives a majority of the votes cast In the event of a tie, election shall be determined by lot.

Section 4. Term of Office.

(a) The president-elect shall be elected for a term of one year and, except in the event of a vacancy in the office of president by death, resignation or total disability, he shall serve for one year as president-elect and then automatically succeed to the office of president.

(b) The recording secretary shall be elected for a term of one year. He may be reelected. He shall not serve in this office for more than two years.

(c) The treasurer shall be elected for a term of three years. He may be reelected. He shall not serve in this office for more than six years.

(d) The term of office of any officer of this Society elected at an Annual Session shall begin with the sine die adjournment of the House of Delegates at such Annual Session.

Section 5. Filling of Vacancies.

(a) In the event of the death, resignation or total disability of the president, the president-elect shall become the president of this Society for the remainder of the year of the president's term of office and for the following year, and the Board of Directors shall elect by majority vote a president-elect to serve for the remainder of the year in which the vacancy occurs. At the next Annual Session following the occurrence of the vacancy in the office of president, the House of Delegates shall elect a president-elect for a term of one year.

(b) In the event of the simultaneous removal during their term of office of both the president and the president-elect by death, resignation or total disability, the recording secretary shall become the president of this Society for the remainder of their term of office. The Board of Directors shall by majority vote elect a president-elect from the slate of nominees for that office from the previous election and a recording secretary from the slate of nominees for that office from the previous election, both of whom shall be elected to serve for the remainder of the year in which the vacancies occur.

(c) In the event of removal from office of the recording secretary by death, resignation or total disability, the Board of Directors shall by majority vote elect a recording secretary from the slate of nominees for that office from the previous election to serve for the remainder of the year in which the vacancy occurs.

(d) In the event of removal from office of the treasurer by death, resignation or total disability, the Board of Directors shall by majority vote elect a treasurer from the slate of nominees for that office from the previous election to serve for the remainder of the term of office for which his predecessor was elected.

ARTICLE IX

Executive Secretary

Section 1. The executive secretary shall be elected by the Board of Directors, shall serve under a written contract, and, subject to the action of the House of Delegates, shall be under the supervision and control of the Board. He shall, under the direction and control of the House of Delegates and the Board of Directors, maintain a central office for the Society in which the records, properties, bonds and legal papers of the Society shall be preserved in properly maintained files. He shall do the administrative work necessary to the operation of the Society and shall be the business manager of the official publication.

Section 2. The executive secretary shall contract for technical exhibits for the Annual Session of the Society and, working with the local chairman of exhibits, shall arrange for all technical exhibits. All receipts from technical exhibits shall go into the treasury of this Society.

Section 3. The executive secretary shall be included as a non-voting member of the Board of Directors and of the Advisory Council.

Section 4. The executive secretary shall distribute to the president, the president-elect and the other officers, and to the committee chairmen and to Advisory Council members, the names and addresses of the officers and committee chairmen of constituent societies as soon after October 1 as their membership status in this Society can be determined, and such lists prepared.

Section 5. An assistant executive secretary, preferably a Medical Technologist (ASCP), shall be appointed by the executive secretary with the approval of the Board of Directors. He shall be salaried, and shall work with and under the direction of the executive secretary so that he may become familiar with all phases of the duties of the executive secretary in the central office of this Society. In the event of the resignation, total disability or death of the executive secretary, the assistant executive secretary

shall maintain the continuity of the functions of the central office of this Society. In such event, he shall serve as acting executive secretary for a period not to exceed one year and shall become executive secretary only upon the approval of the Board of Directors.

ARTICLE X
Board of Directors

Section 1. The Board of Directors shall consist of the president, the president-elect, the recording secretary, the treasurer, the immediate past-president and six members of this Society elected by the House of Delegates.

Section 2. The Board of Directors shall represent the Society when the House of Delegates is not in session and only when urgent business necessitates immediate action that cannot wait until the House of Delegates reconvenes. In no case, except in an emergency, shall the Board of Directors transact business that vitally affects the interests of this Society and in no case shall the Board of Directors take any action which binds the Society beyond the next ensuing Annual Session, except as specific authority for such action has been previously granted by the House of Delegates.

Section 3. Any person who is, and for at least three years prior to election or appointment has been, an active member of this Society shall be eligible to serve on the Board of Directors.

Section 4. Method of Election.

(a) Two members of this Society shall be elected annually to the Board of Directors by the House of Delegates each to serve for a term of three years.

(b) The Nominations Committee shall prepare a list of at least four nominees to fill the two vacancies on the Board. Nominations may be made from the floor. The qualifications of all persons nominated from the floor shall be presented to the House of Delegates at the time such nominations are made. Elections shall be by ballot and a majority of votes cast shall be necessary for election. If more than two candidates receive a majority, the two receiving the highest number shall be elected. In the event that no candidate receives a majority, the candidate with the lowest number of votes shall be eliminated, and the balloting shall continue until a candidate receives a majority vote. In the event that only one candidate receives a majority on the first ballot, he shall thereupon be declared elected, and balloting shall continue on the remaining list of nominees. In the event of a tie, the candidate to be elected or the candidate to be eliminated shall be determined by lot.

(c) Election shall be conducted by the elections committee.

Section 5. The term of office of any member of the Board of Directors elected at an Annual Session shall begin with the sine die adjournment of the House of Delegates at such Annual Session.

Section 6. The president of this Society shall serve as chairman of the Board of Directors and the recording secretary shall serve as secretary of the Board.

Section 7. The president or president-elect and seven other voting members of the Board shall constitute a quorum.

Section 8. The annual meeting of the Board of Directors shall be held within two weeks prior to the first meeting of the House of Delegates at each Annual Session, the time and place of such meeting being designated by the chairman.

Section 9. In the event of removal by death, resignation or total disability of a member of the Board of Directors who is not an officer of this Society, the president of this Society shall appoint one of the candidates from the slate of nominees for members of the Board of Directors from the preceding election to serve until the next Annual Session. At the next Annual Session the vacancy shall be filled by election by the House of Delegates.

Section 10. The method of carrying on the business of the Society between the regular annual or interim meetings of the Board of Directors shall be by mail as herein provided.

(a) The material shall be submitted in writing in two copies, one to the president, the second to the executive office for filing. Full reasons for the

proposed action shall be stated, and the proposal shall be accompanied by the second of another member of the Board.
(b) Within fifteen days of receipt of material, the president shall submit the proposal, accompanied by the reasons, to the executive office with instructions that it be sent to the Board of Directors for their approval and returned to the executive office.
(c) The results of the vote of the Board of Directors shall then be sent to each Board Member by the executive office.

ARTICLE XI
House of Delegates

Section 1. The membership of the House of Delegates shall consist of the members of the Board of Directors, the members of the Advisory Council and the delegates elected by their constituent societies or appointed in accordance with the provisions of these Bylaws.

Section 2. Each constituent society shall hold an annual election for the purpose of the election of delegates to represent the members of said constituent society at annual or special sessions of the Society.

Section 3. (a) The basis of representation is as follows: one delegate and one alternate for each 25 active members, or major fraction thereof of the constituent society. When the membership of a constituent society exceeds 250 active members, the basis of representation shall be one delegate and one alternate for each 50 active members, or major fraction thereof, above 250.

(b) The number of delegates to which each constituent society is entitled shall be determined by the number of active members who have paid current dues to and are in good standing with this Society. A member who has transferred from one constituent society to another shall be counted as a member of the geographical area in which he holds his membership thirty days prior to the opening of the Annual Session.
(c) Notwithstanding anything to the contrary, each constituent society shall be entitled to at least one delegate, and no delegate or delegates representing a constituent society shall have a greater number of votes than the total membership of said constituent society.

Section 4. The secretary of each constituent society shall send to the executive secretary of this Society at least two weeks before the opening of the Annual Session a list of delegates and alternates from his society with their addresses and registry numbers or academic degrees.

Section 5. If the combined number of delegates and alternates present at any Annual Session from any one constituent society is less than the quota of delegates from that society, the president of that constituent society, or in his absence the other officers of that society, shall fill the quota from eligible active members of the constituent society present at the Annual Session. When necessary the president of this Society shall name these delegates.

Section 6. Each delegate and each alternate shall present his credentials to the chairman of the convention credentials committee when he registers at the Annual Session.

Section 7. The members of the Board of Directors and of the Advisory Council and the officers of this Society shall not be included in the list of elected or appointed delegates from a constituent society to the House of Delegates.

Section 8. Delegates and alternates shall serve from the time of the first meeting of the House of Delegates at the Annual Session for which they were elected as delegates and alternates to the first meeting of the House of Delegates at the next ensuing Annual Session.

Section 9. A quorum of the House of Delegates shall be a majority of its accredited members registered at the Annual Session.

Section 10. Any member in good standing in this Society is eligible to attend the annual meetings of the House of Delegates and to participate in discussion but the privilege of the floor shall be granted to a non-delegate only by consent of the House. Only members of the House of Delegates may vote.

ARTICLE XII
Advisory Council

Section 1. (a) The Advisory Council of this Society shall consist of those persons who are the presidents and presidents-elect of the constituent societies or, in the absence of a president-elect such other person as may be selected by the constituent society, and those persons who are the members of the Board of Directors of this Society as of the day immediately following the sine die adjournment of the House of Delegates at any Annual Session. These same persons shall remain on the Advisory Council until the sine die adjournment of the House of Delegates at the next ensuing Annual Session even though in that period the presidents of constituent societies on the Council have become the past presidents and the presidents-elect have become presidents of their respective constituent societies.

(b) With the exception of the members of the Board of Directors, it shall be the privilege of a member of the Council to appoint any other active member of his constituent society as his alternate when he is unable to be present at a meeting of the Council, provided that only two active members from a constituent society shall be allowed certification as members of the Advisory Council at any one Annual Session of this Society.

Section 2. The purpose of the Advisory Council shall be to consider matters of importance pertaining to the interest and welfare of the members of this Society and of the members of the profession of medical technology in general. The Advisory Council shall have no power to act for the Society. It may make recommendations to the House of Delegates. By means of a report made by the chairman or other duly authorized member of the Council to the House of Delegates it may bring matters of importance to the attention of the House of Delegates.

Section 3. Officers of the Advisory Council shall consist of a chairman, a vice-chairman, a recording secretary, an assistant recording secretary and a corresponding secretary.

(a) Officers of the Advisory Council shall be elected at a meeting following soon after the sine die adjournment of the House of Delegates at each Annual Session.

(b) No member of the Board of Directors shall hold office in the Advisory Council.

(c) The retiring chairman of the Advisory Council shall call a meeting of the Advisory Council soon after the sine die adjournment of the House of Delegates for the purpose of organizing the Advisory Council. He shall act as chairman pro tem of this meeting. He shall read, or distribute in writing, to the members of the Council a list of the persons eligible for office. Nominations shall be made from the floor. Election shall follow. A majority vote of all members of the Advisory Council present at that meeting of the Council shall elect.

(d) A quorum of the Advisory Council shall be a majority of its members.

(e) Officers shall take office immediately after their election and they shall remain in office until the sine die adjournment of the House of Delegates at the next ensuing Annual Session.

Section 4. The duties of the officers of the Advisory Council shall be as follows:

(a) The chairman shall preside at all meetings of the Council. He shall provide for the preparation and presentation of reports and recommendations from the Advisory Council to the House of Delegates.

(b) The vice-chairman shall in the absence of the chairman assume the duties of the chairman.

(c) The recording secretary shall take the minutes of the meetings of the Advisory Council and shall keep a permanent record of these minutes. He shall, within thirty days after the close of the Annual Session, prepare and distribute to the members of the Advisory Council a copy of the minutes of the meetings held by the Council at the time of the Annual Session just closed.

(d) The assistant recording secretary shall in the absence of the recording secretary assume the duties of the recording secretary.

(c) The corresponding secretary shall carry on the correspondence for the Advisory Council.

Section 5. Any member in good standing in this Society shall be eligible to attend the meetings of the Advisory Council and to participate in discussion, but the privilege of the floor shall be granted to a non-member of the Council only by consent of the Council. Only members of the Council may vote.

(a) Each constituent society shall be entitled to two and only two voting members at any meeting of the Council.

Section 6. The routine operating expenses of the Advisory Council shall be included in the annual budget of this Society. Any request for an appropriation for special activities of the Council shall be presented to the Board of Directors for approval.

ARTICLE XIII

Representation on the Board of Registry

Section 1. The president, the president-elect, the immediate past president and one member-at-large of this Society shall represent the Society as members of the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists.

Section 2. The member elected at large shall serve five consecutive years, the first member at large to be elected at the June 1960 meeting of the House of Delegates. This person shall have the same qualifications as stated in Article XIV, Section 2. Nominations shall be made by the Nominations Committee or from the floor in the same manner as stated in Article VII, Section 3 (b). Election shall be by ballot.

Section 3. The representative at large shall not serve more than five years except if appointed to fill a vacancy.

Section 4. In the event of death, resignation or total disability of this representative at large to the Board of Registry, the Board of Directors shall fill the vacancy by appointment until the next House of Delegates meeting where the vacancy will be filled by election for a five year term.

ARTICLE XIV

Representation on the Board of Schools

Section 1. Four representatives of this Society shall be elected by the House of Delegates to serve on the Board of Schools of the American Society of Clinical Pathologists. Nominations shall be made by the Nominations Committee or nominations may be made from the floor provided qualifications of persons so nominated shall be presented at the time such nominations are made. Election shall be by ballot. A majority of votes cast elects.

Section 2. Persons who are, and for at least five consecutive years immediately prior to election or appointment have been, active members in good standing of this Society shall be eligible for election to the Board of Schools provided, however, that such persons shall hold academic degrees at least at a baccalaureate level and have had at least three years of teaching experience within the current decade in a school of medical technology approved by the Council on Medical Education and Hospitals of the American Medical Association and that they shall have held office either in this Society or in one of its constituent societies or have held membership on the Board of Directors of this Society or on a comparable Board of a constituent society.

Section 3. A representative to the Board of Schools shall be elected to this position for a term of four years. One representative shall be elected at each Annual Session. At the Annual Session in 1960, the terms of each of the present elected representatives shall be extended one year.

Section 4. A representative to this Board shall not serve for more than two terms or more than eight consecutive years.

Section 5. In the event of the death, resignation or total disability of a representative of this Society to the Board of Schools, the Board of Directors shall fill the unexpired term by appointment.

ARTICLE XV

Elections Committee

All elections by the House of Delegates shall be conducted by an Elections Committee consisting of five delegates appointed by the chair. Each member of the elections committee shall receive and carry out the list of instructions previously prepared by the chair in accordance with the parliamentary authority of the Society.

ARTICLE XVI

Special Committees

Section 1. There shall be a Nominations Committee composed of seven active members of this Society, no two of whom shall be from the same constituent society and none of whom shall be an officer of this Society.

(a) The House of Delegates shall elect the Nominations Committee. Nominations for membership on this committee shall be made only from the floor of the House. The time for these nominations shall be fixed for the first day of the meetings of the House of Delegates at each Annual Session, and each constituent society shall be entitled to make only one nomination. The time for election of the Nominations Committee shall be fixed for the second day of the meetings of the House of Delegates at each Annual Session.

(b) Election shall be by ballot, and each delegate shall be provided with a printed ballot bearing names of all nominees. The seven persons receiving the highest number of votes shall be considered elected. The person receiving the highest number of votes shall serve as chairman of the committee.

(c) In the event of a vacancy due to death, resignation or refusal to serve, the Board of Directors shall elect by majority vote an eligible member of this Society to fill the vacancy. The remaining nominees for this committee shall be considered first, in the order of the highest number of votes received.

(d) The Nominations Committee shall provide for the publication in the September-October issue of the official publication of this Society the names and offices of those officers, directors, and representatives for the Board of Schools and Board of Registry of Medical Technologists of the American Society of Clinical Pathologists whose terms of office expire at the next Annual Session.

(e) It shall solicit from the constituent societies or from any active member of this Society, names and qualifications of qualified candidates for such offices and positions, with instructions that such material be in the hands of the Nominations Committee not later than December 1st. Only the names of members who have consented to serve if elected shall be submitted by a constituent society or by a member of this Society to the Nominations Committee.

(f) The Nominations Committee shall prepare a slate of at least two nominees for each office and other position to be filled by the House of Delegates. Not less than sixty days before the date of the next Annual Session, a list of nominees together with their qualifications shall be distributed to each active member of this Society.

Section 2. Other special committees may be appointed at any time by the president on authority given him by the House of Delegates or the Board of Directors.

Section 3. The president shall appoint the necessary personnel for committees that shall arrange for the Annual Session of this Society.

Section 4. The President shall appoint a Minutes Committee with authority to approve and correct the minutes of the previous Annual Session of the House of Delegates. An abstract of these minutes and of the minutes of the meetings of the Board of Directors held since the first meeting of the House of Delegates of the last Annual Session shall be published and distributed to members not less than 60 days prior to the next Annual Session.

ARTICLE XVII

Standing Committees

Section 1. There shall be the following ten standing committees: Constitution & Bylaws; Education; Finance; Legislation; Membership; Public Relations; Recruitment & Vocational Guidance; Research; Scholarship; Standards & Studies. Each of such committees shall be composed of six members, with such geographical distribution as shall be conducive to effective work.

Section 2. At the beginning of his term of office the president of this Society shall appoint, each for a term of three years, two members to each of the standing committees to succeed the two members whose term ended with the sine die adjournment of the House of Delegates at the Annual Session. The president shall appoint a chairman for each standing committee for a term of one year. A member may not serve for more than six consecutive years on the same standing committee. Only active members may be appointed to membership on standing committees.

Section 3. The duties of the standing committees shall be as follows:
(a) The Constitution and Bylaws Committee shall:

(1) Examine all proposed amendments to the Bylaws of this Society. Proposed amendments to the Bylaws of this Society may be submitted by an active member of this Society or by a constituent society. After the committee makes such changes as are necessary to put these proposed amendments in proper form without altering their intent, it shall have them distributed to each active member of this Society not less than sixty days prior to the next ensuing Annual Session of this Society.

(2) Thirty days after the adoption of an amendment or amendments to the Articles of Incorporation or Bylaws of this Society, the recording secretary shall send an official report of such adoption to the executive secretary who shall provide for publication of the announcement of adoption of such amendments in the September-October issue of the official publication of this Society.

(3) The Constitution & Bylaws Committee shall examine the proposed constitution, or Articles of Incorporation, and bylaws of any group petitioning for a charter from this Society and shall bring a report and a recommendation concerning the chartering of such group to the House of Delegates at the next ensuing Annual Session of this Society.

(4) The Constitution & Bylaws Committee shall examine all proposed amendments to the constitution and bylaws of a constituent society. It shall submit the same to the chairman of the Board of Directors with an opinion only if a majority of the committee members disapprove the proposed amendments. In such case these must be approved by a majority of the Board of Directors before the proposed amendments are presented to the membership of the constituent society to be voted on in the manner prescribed by the bylaws of said society.

(b) The Education Committee shall develop teaching aids, encourage the establishment of laboratory libraries, prepare and make available to members and to constituent societies current lists of visual aids, study the curricula of approved schools of medical technology, make suggestions and receive such from the Board of Schools, and aid graduate and undergraduate students of approved schools of medical technology in every way possible.

(c) The Finance Committee shall prepare the Annual Budgets of the Society for the Treasurer's Account, the Executive Office Operation Account and THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY Account. This committee shall approve special project expenses as may be required by any of the standing or special committees of this Society, and at the request of the president shall approve requests for funds for such expenditures as are not specifically provided for in the budgets. At the request of the president and/or Board of Directors, this committee shall carry on any other activities relating to the financial matters of this Society.

(d) The Legislation Committee shall assume the duties assigned to it by the House of Delegates and Board of Directors.

(e) The Membership Committee shall plan ways and means of cooperating with the membership committees of constituent societies with the aim of increasing the membership of this Society and of the constituent societies.

(f) The Public Relations Committee shall, by dispensing information and promoting public understanding of the profession of medical technology, act as a liaison group between this Society and other professional or lay groups.

(g) The Recruitment & Vocational Guidance Committee shall have the responsibility of recruiting students into the field of medical technology.

(h) The Research Committee shall direct the research activities of this Society.

(i) The Scholarship Committee shall have the responsibility for compiling and publicizing information regarding scholarships, fellowships and student loans for ASCP Registered medical technologists and for students who are studying, or plan to begin the study of medical technology under a curriculum which will qualify them for ASCP registration; for answering inquires regarding these or other sources of financial aid for education in the field of medical technology, or one of the specialties in the field of medical technology. This committee shall have also the responsibility for developing such additional scholarships and student loans as may be desirable for ASCP Registered Medical Technologists and for students now studying, or planning to study under a program which will qualify them for ASCP Registration; for the activities of the scholarship committees of affiliated state societies; and carrying on such other activities as may be deemed necessary to meet the scholarship needs of those who are now working or studying in the field of medical technology and those who are planning to enter the field of medical technology.

(j) The Standards and Studies Committee shall study medical technology ethics, economics, standards and other kindred problems. This committee shall inform the members of this Society of the activities of other professional medical technology organizations. It shall promote better understanding of professional medical technologists by the public so that the public may discriminate between adequately and inadequately trained members of our profession.

ARTICLE XVIII
Fiscal Year

The fiscal year shall be from July 1 to June 30, inclusive.

ARTICLE XIX
Official Publication

Section 1. The official publication of this Society shall be THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY, the name of which is patented and the contents of which shall be copyrighted.

Section 2. The official publication shall be made available to all active, affiliate, corresponding and student members of this Society who have paid their dues and are in good standing, and to all honorary members.

Section 3. The editor of the official publication shall be elected by the Board of Directors and shall serve under written contract.

Section 4. The Editorial Board shall consist of the editor, two or more advisory editors and two or more associate editors. The advisory and associate editors shall be appointed annually by the editor with the approval of the Board of Directors.

Section 5. All editorials shall be approved by the editor-in-chief and one other member of the Editorial Board before publication.

Section 6. All papers read at convention by members of this Society or submitted to the Society shall become the property of the Society and shall be published, if warranted, in THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY under the copyright of the Society unless a petition for the privilege of prior publication has been obtained of the Editorial Board.

ARTICLE XX

The excutive office of the Society shall be located at Houston, Texas.

ARTICLE XXI

Parliamentary Authority

Robert's Rules of Order Revised shall govern the business proceedings of this Society except when otherwise specified in these Bylaws.

ARTICLE XXII

Amendments

Section 1. The Bylaws of this Society may be amended as follows:

(a) Any proposed amendment to these Bylaws recommended by either a constituent society or by an active member of this Society shall be submitted in writing to the chairman of the Constitution & Bylaws Committee not later than December 1st. Twelve copies of the proposed amendment in context shall be submitted. The Constitution & Bylaws Committee shall make such changes as may be necessary to provide proper form without altering the meaning of the amendment. After such changes have been made the proposed amendment shall be distributed to each active member of this Society not less than sixty (60) days prior to the opening of the next Annual Session, at which Session the proposed amendment shall be presented to the House of Delegates for vote.

(b) Amendments to these Bylaws shall be adopted by a two-thirds vote of the House of Delegates, except that a proposed amendment to Article XX of these Bylaws shall be approved by a two-thirds vote of the Board of Directors before it may be submitted to the House of Delegates for vote.

Section 2. A complete revision of these Bylaws shall be presented to a meeting of the House of Delegates at an Annual Session only upon two-thirds vote of the House of Delegates at one Annual Session ordering a revision of the Bylaws for presentation to a meeting of the House of Delegates at the next ensuing Annual Session. A two-thirds vote of the House of Delegates shall be necessary for adoption of a revision of these Bylaws.

Section 3. Manner of voting on amendments to Articles of Incorporation:

(a) Amendments shall be made only at an Annual Session of the House of Delegates by a majority vote of the total number of its accredited members. The vote shall be by roll call.

(b) In the event that the authorized quota of accredited delegates of any constituent society is unable to be present at the Annual Session of the House of Delegates, such constituent society shall be entitled to send the duly certified votes of its absent accredited delegates to the executive secretary 10 days before the date of said Annual Session, such votes to be cast at the time the roll call vote is taken.

COMPARISON OF MACRO AND MICRO SEDIMENTATION RATES*

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Principles

This article is written solely to give some idea as to the relationship of the Wintrobe macro and Aloe micro sedimentation rates. Many doctors have requested from our laboratory the relationship of these two methods but we had none. In a series of 150 tests, a method was found that could be used with a degree of reliability (taking into consideration a $\pm$ 4 mm. variation).

"The principle of a sedimentation rate is due to changes in the surface charge of erythrocytes which cause them to aggregate. These charges are related to alteration in the plasma, particularly in the physical state of the plasma colloids." (Wintrobe[1]).

Reagents and Equipment

1. CBC oxalate tube with potassium ammonium oxalate and heparin (Macro).
2. The Aloe Company kit including 5 per cent sodium citrate complete for Micro.[2]
3. Wintrobe tubes with racks.
4. Interval timer.
5. Correction chart for the macro method by Wintrobe and Lansberg.

Procedure

a. Draw approximately 5 cc of blood from venipuncture and place a large amount on a clean slide; the remainder in a CBC tube.
b. Set up micro method as per instructions of the Aloe Company, using the sodium citrate as the anticoagulant in duplicate[2].
c. Set up immediately after, the macro sedimentation test, using the Wintrobe method in duplicate. Correct with chart.
d. Set up hematocrits in duplicate.
e. Read results of both methods after one hour.
f. Be careful to keep everything at a constant temperature, and use the same time and technique.
g. The blood was taken at the time of routine venipuncture; thus a random sampling was obtained with no particular group of patients being studied.

Results

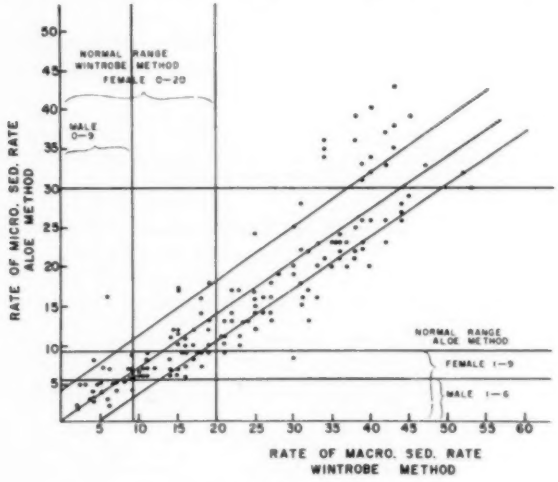
Many of the sedimentation rates were very near, if not precise, duplicates; both in the micro and the macro methods. When there was any slight variation, the lowest sedimentation rate was chosen because there is more reason for increasing a sedimentation rate than in decreasing one.

a. A very precise relationship existed between the two methods, based on a 0.88 correlation index. A correction factor on the micro sedimentation rate was not used because there was none that could be

* Read by title 27th Annual Convention of ASMT, Phoenix, Arizona, June 1959. Received for publication, January 1959.

found. However, the correlation index is 0.91 when the micro is corrected using the Wintrobe chart. The 95 per cent Confidence Interval for the Population Slope is .512—.592 for the corrected micro, and .623—.740 for not correcting. Therefore, on this basis, it is felt that using the correction factor is permissible, and the results are reliable and definitely not chance.

b. By using per cents the comparison is also shown (see Graph A).



Graph A
Using The "Uncorrected" Micro. Sedimentation Rate

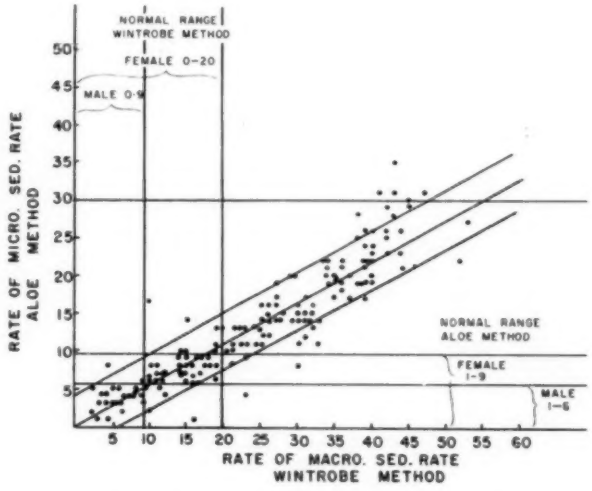
Using the uncorrected micro, and taking the Best Fit Straight Line through X = 23.64, Y = 16.05, and then taking into consideration a ± 4 variation, we find that 73 per cent (109) fell inside these boundaries, and that 27 per cent (41) fell outside. Of this 27 per cent, we see that 13 per cent (19) were on the left side and that 14 per cent (22) were on the right. Going still further in the breakdown as to ranges (using 10 — 15 = Slight increase, 16 — 30 = Moderate increase and 31 + = High increase) this left side of 13 per cent comprises 46 per cent of all that fell outside the boundaries, and the right side 54 per cent. See Table A for further breakdown.

TABLE A

Sides	Total Points and Percents	RANGES		
		High Inc.	Mod. Inc.	St. Inc.
Right	22 (14%)	0 (0%)	9 (6%)	13 (8%)
Left	19 (13%)	12 (8%)	6 (4%)	1 (1%)
Total's	41 (27%)	12 (8%)	15 (10%)	14 (9%)

This Table shows in percentages where the points outside the boundaries on graph A fall. The most sedimentation rates outside the 95% confidence interval being about even on both sides.

c. By using the correction factor in micro method (see Graph B) and finding the Best Fit Straight Line passing through the points X = 23.63, Y = 13.04, we now find that 88 per cent (132) fall within the boundaries, and only 12 per cent (18) outside. These also fall into two categories. The left side 7 per cent (11), or 61 per cent of all that fell outside, and the right side 5 per cent (7), or 39 per cent of the total outside. See Table B for the further breakdown of this graph. By correcting the micro sedimentation with the Wintrobe charts, better results were found.



Graph B
Using The "Corrected" Micro. Sedimentation Rate

TABLE B

Sides	Total Points and Percents	RANGES		
		High Inc.	Mod. Inc.	St. Inc.
Right	7 (5%)	0 (0%)	1 (1%)	6 (4%)
Left	11 (7%)	4 (3%)	5 (3%)	2 (1%)
Total's	18 (12%)	4 (3%)	6 (4%)	8 (5%)

This Table shows in percentages where the points outside the boundaries on graph B fall. The most sedimentation rates outside the 95% confidence interval being about even, but less than graph A.

The Gothenburg Hospital for Children[3] uses the micro method (Linzenmeier-Raunert) solely and found good results, but state that one of the primary causes for error was a clot causing a decreased sedimentation rate. They, as well as I, have found no real significance between venous and capillary blood; also no difference between oxalates. Also, a 5 —6 mm. variation in micro methods was found, and the high rates had greater variation in comparison with the macro method.

The preceding graphs give an approximate relationship of the methods. A good relationship of the micro to macro methods can be found by using either one if you feel that the correction is not reliable.

REFERENCES

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CURRENT PROBLEMS OF BLOOD BANKING*

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The title of my paper is a general one and may appear to be vague and prosaic to many of you. At times it is important for all of us to pause, take stock of our present situation and to assess future trends. In looking over the program of the meeting, therefore, I decided it might be well to digress from the more technical and scientific aspects of blood banking and instead to discuss two blood bank problems which are becoming increasingly troublesome and which will soon require solution.

PART I

First, the present supply of blood, in my opinion, will be inadequate to meet the future demands of medicine and surgery. We need only to look about us and to compare the amount of blood which is being used today in operations with that before World War II, or even for that matter, with that of ten to twelve years ago, to realize the great difference. Medical science is advancing so rapidly that expansion of the supporting and ancillary services to keep pace with scientific progress is becoming increasingly difficult. It is altogether likely that after a time even more extensive operations will become technically feasible, provided the supply of blood is adequate. An acceptable substitute for whole blood is not yet in prospect.

As you all know, blood is procured from persons who are replacing the blood needs of friends or relatives, who are acting as professional donors, or who are entering into a contract with an agency which in return for donation of blood will insure the donor and his family for a specified period of time. Is it likely that a greater number of persons in each category can be induced to become blood donors than is presently the case? In regard to the first category, I am inclined to doubt whether the supply of persons can be greatly augmented. If blood must be replaced from among the friends and relatives of a patient who has undergone an operation requiring much blood there is obviously a limit to the size of families and circles of friends which most persons may have. The greater the number of persons in a given community who require large amounts of blood, the greater the overlap among their friends and relatives. Thus, most persons have relatives and friends in common with other persons in the community. This overlap imposes definite limitations on the expansion of the voluntary replacement donor pools. Some patients, furthermore, must travel long distances to medical centers for complex operations. Blood replacement by friends and relatives under these circumstances may be very difficult. Although preventive medicine may be expected to reduce some of the need for present day operations, one may predict that operations to correct lesions of the heart and blood vessels will increase in the older age group of patients. Old persons are those who are least likely to have sufficient friends who are physically qualified to donate blood. Even at present

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there are many elderly patients in hospitals who can obtain neither enough donors nor enough funds to meet their obligations for blood. From these considerations, I fear that persons who have no insurance coverage for blood will have increasing difficulty in personally finding replacements for their blood needs following the medical or surgical condition which requires transfusions.

In regard to professional donors, the second category, we have been able to persuade only about 10 per cent of replacement donors with the less common blood groups to register on a professional donor list. Our blood situation is fortunate in that we have available university personnel who are the mainstays of our list of registered professional donors. The other common source of professional donors in larger communities is impoverished men of the lower economic and social groups. The number of such donors is also limited. Donating blood is an unpleasant experience for many and there is little chance, I think, that an appreciably greater proportion of persons will be inclined to serve as professional donors in the future unless inducements of one sort or another are greater.

It is the contract or insurance type of program which seems to me to give the greatest promise of increasing the donor supply in the future. One of the objections to becoming a member of a registered donor list has been the need for calling on these persons for donation several times a year. In the case of insurance programs one or at most two donations a year are all that is required. Second, the broader the base of insured donors, the more blood will be available for the general supply and the more evenly distributed in both time and place will be the supply. A common practice now in some communities is to insure at the rate of two pints for each three donated. This excess is considered sufficient to cover the cost of operation and to furnish an excess of blood which may be used for emergencies and in cases in which the uninsured patient is unable to meet his blood obligations. Thus, it seems to me that it is incumbent on all of us interested in blood banking to give our whole-hearted aid and support to those organizations which are engaged in such donor insurance activities. There is not, however, a single organization which is meeting the blood procurement needs throughout the nation.

At present the American National Red Cross through its local chapters and blood centers is engaging in a nation-wide blood insurance program but this activity even though geographically extensive is not by any means supplying the total blood needs of the nation. Parallel to this activity, the American Association of Blood Banks has been engaging in a national program through sponsorship of the National Clearing House for the exchange of blood credits. Blood itself may also be exchanged under certain circumstances. Although rendering a valuable service, this agency is not yet so effective as to replace the functions of the Red Cross or to provide a nation-wide blood replacement program. Meanwhile the Joint Blood Council has recently entered the picture. This is an organization which is composed of representatives of five national societies: American Association of Blood Banks, American Hospital Association, American Medical Association, American National

Red Cross, and the American Society of Clinical Pathologists. According to available information the Joint Blood Council was established in 1955 as a voluntary non-profit organization to promote blood banking in the United States by providing an advisory and policy-making service on a national level. In view of the present lack of an effective nation-wide blood procurement program, the Executive Vice-President of the Joint Blood Council has recently been urging its member organizations to authorize the Joint Blood Council to undertake such a nation-wide program on an operating basis. At the annual meeting of the American Association of Blood Banks in November, 1959, however, the Board of Directors strongly recommended that the Joint Blood Council continue on a non-operating basis. Concern about the lack of an effective national program has been voiced by the Armed Forces, by the U. S. Public Health Services Division of Health Mobilization and by various labor unions. Thus, although at least three organizations are actively interested in or actually engaged in operating a national blood program, none has been completely effective in providing a service which is satisfactory. To obtain the desired result, two major difficulties must first be surmounted. The first is of course how will sufficient blood be obtained on a national basis, the second is who will administer the program.

As I have already stated, it is my conviction that the best solution, if not the only one, to the first question is a blood insurance program. It is true that the American people are now being insured to such an extent in various aspects of their lives and deaths that they may well feel a revulsion to another project for insurance. Insurance, furthermore, is taking an ever larger fraction of the family budget. But we are talking about blood which is given to the insured in return for blood which has been donated by the insured as an insurance premium and not about transfusions given to the insured in return for premiums paid in money. If the practice of insuring against blood needs by payment of monetary premiums, now available in some areas of the country, becomes widespread, it should be obvious to all that the supply of blood itself will be diminished to an alarming extent and will be limited to the small number of persons who are willing and able to serve as professional donors.

In regard to the second question, I believe that the public will soon demand a speedy solution on a national scale. This need for a solution will become more and more apparent to individual persons as well as to such organizations as labor unions and various branches of the federal and state governments as the difficulties of supply and replacement are continually increased by the use of even more complicated surgical procedures. There is already enough experience with insurance and credit programs on a regional basis, I believe, to make the solution of the problem relatively easy if we can decide what agency or what type of agency will most effectively administer the program. Thus, the real problem is who should be entrusted with this responsibility.

By priority, precedent, and scope of activity, the American National Red Cross merits serious consideration for this role. It is a non-profit and non-government organization which has great national prestige and

which can always be relied upon in time of either local or national emergency to give invaluable service to the injured and the needy. The Red Cross blood insurance and replacement program already extends over much of the nation. Why has the Red Cross not already filled the need for a national blood program and why is it handicapped to fill this need in the future? Although I have no more than public knowledge of the charter of the Red Cross and the general policies of its Administration, it should be obvious to all that the Red Cross is not permitted to accept payment for services. Financial support is obtained by a yearly appeal to the public for monetary donations. Such donations are solicited on the basis of the over-all functions of the Red Cross and not specifically on the basis of the blood program. Even though supplemented by the great personal contributions of unpaid community workers, the funds allocated for the blood program in my opinion appear to be inadequate to expand the function to cover the blood needs of the nation. In support of this opinion can be cited the recent policy of the Red Cross in making an equipment charge for each pint of blood issued. By this means it appears that funds derived from voluntary contributions can be conserved for other expenses concerned with the blood program. If the Red Cross is to expand its personnel and blood collecting and issuing activities in order to satisfy all of the blood requirements of the Nation, it seems to me to be imperative that Red Cross Chapters be permitted to sustain their local activities by means of service charges. These charges need not be exclusively monetary but may be also payment in kind, i.e. more blood donated than transfused to the insured. Some persons prefer to pay for transfusions rather than donate blood to an insurance program. If an excess number of units is collected, blood may go to them in return for a penalty charge, as well as to persons who are unable to obtain this type of insurance. This suggestion also entails a second alteration in the Red Cross policy that the transfusing agency is reimbursed for blood administered on no more than a one for one basis. By accepting blood as a part of the service charge this ratio could be increased by a fraction to cover the five to fifteen per cent losses which community blood banks suffer from outdating, contamination, accidental breakage, etc. Additional funds will also be required for the Red Cross to develop an inspection and accreditation program for blood banks. Unless these proposed policy changes can be made, it seems to me highly unlikely that a nation-wide blood program can be satisfactorily carried out by the Red Cross.

A second organization which lately appears to be qualified for consideration as the administrator of a national blood program is the American Association of Blood Banks. This association is likewise a voluntary non-profit organization which is supported by dues and contributions. It has sponsored a National Clearing House for blood which is now in operation in all of the five districts of the United States. In some areas the activities have been largely concerned with a transfer of credits, but exchange of blood as well as credits is being actively promoted. It is expected that exchange of blood will become an ever more important feature of the program as more blood banks become accredited by the Association. Inspection and accreditation of blood

banks is absolutely essential to safe operation of a national program entailing the exchange of blood as well as credits. In my opinion no program will be effective unless blood can be freely exchanged. Financially this program has been set up on a pay as you go basis. That is the operation is on a fee for service basis. A minimum of support has been needed in the early phases from organizations such as state and county medical societies. Transaction and service charges make the Clearing House Districts self-supporting without undue cost to patient or hospital. The American Association of Blood Banks rightly hopes that the program will in time fill the blood needs of those areas in which the Red Cross is not operating regional blood centers or is unable to supply all of the blood which is requested. The AABB program will not fulfill its expectations, however, unless the public gives its enthusiastic and continuing support.

The third agency which has expressed interest in a national blood program is the Joint Blood Council. Although deriving support from the five national societies already named and although its backing has therefore a wide medical and administrative base, the Joint Blood Council has no operating personnel. To undertake a national blood program would entail a very large expenditure. Much time would be required for newly employed personnel to gain the necessary training and experience before the program could be effective. The time required, as well as the cost, would in my opinion be prohibitive if this program is to be supported by other means than the Federal Government. I think the societies which are represented on the Joint Blood Council would be better advised to lend their support to the National Clearing House and the American Association of Blood Banks inspection and accreditation programs which now are operating and which would require much less expenditure in time and money than the establishment of a new program.

Some persons doubt that any voluntary organization, be it Red Cross or the American Association of Blood Banks, can ever hope to have a really adequate blood program which will be so effective on a national scale that virtually all blood needs will be satisfied everywhere in the nation. This is not an unreasonable doubt. Our society is so complex and local customs and policies are so diverse that coordination of any national voluntary program is subject to great difficulties. Furthermore, it is argued, that no voluntary program can be entirely satisfactory. There are always persons who lack the foresight or the willingness to insure themselves against emergencies and disaster. Also, the aged and the infirm are often unable to obtain blood insurance by blood donation. The proponents of compulsory health insurance are using much the same arguments against the Blue Cross-Blue Shield programs in favor of the administration by an agency of the Federal Government. The question is posed, therefore, whether we would not all be better off by scrapping the Red Cross or any other voluntary blood program in favor of a government program which of course would be supported by taxes. As I see it, the answer is no, for a simple reason which to me is more important than any other. We are used to paying taxes in money, but we are not used to paying taxes in blood. It is true that in Great Britain nearly all of the required blood is obtained on a voluntary basis through

a government operated national blood program as a part of the National Health Services. It must be remembered, however, that Britons have been indoctrinated in obligations pertaining to community health problems over a long period of time through their government operated National Health Services and through their war experiences, factors to which the American people have not as yet been exposed. The average Briton is also more observant of rules and regulations than his American counterpart, as shown by his orderliness in "queuing up" for buses or trams in contrast to our own custom of pushing ahead and shoving for buses and subways. Americans can be depended upon in time of war or great national emergency, but in more normal times I am doubtful that enough blood will be donated until the people have been better indoctrinated concerning this responsibility. Blood donations obviously cannot be enforced as can monetary taxation and so the success of a government sponsored program will also depend upon voluntary contributions of blood just as a non-government program will do. This question, therefore, also remains to be answered.

PART II

The second great problem is whether an adequate supply of qualified personnel will continue to be obtainable in view of the moral and legal responsibilities which must be assumed by blood bank personnel and practicing physicians.

There is perhaps no branch of laboratory medicine in which the moral responsibility is greater than in blood banking. In my opinion the responsibility is even greater than in the case of the surgical pathologist whose diagnoses so frequently are critical in the care of the patient. The surgeon has the privilege of disagreeing with the pathological diagnosis, but he has not the time or facilities to check on the accuracy of a cross-match for blood transfusion. The moral responsibility for the transfusion thus becomes a heavy burden on blood bank personnel. Physicians and technicians have a continuing obligation to exercise the utmost care and accuracy in both the technical and administrative phases of the work. Because of this responsibility, in my opinion, the director of a blood bank should be a physician licensed to practice medicine in that particular state in which the blood bank is located. Furthermore the physician should be fully aware of his responsibilities and of course should be meticulous and conscientious in exercising them. He must insure that his technical associates are well versed in techniques and are competent and trustworthy. Now I am sure you have already asked yourselves the question whether a physician should personally interview each blood donor and whether he should personally inspect each typing and cross-match. In small hospitals in which blood banking is not a large operation and in which the physician-pathologist is usually in the laboratory when blood is being cross-matched, it is desirable, except in emergencies, that he should do so. Personal checks of this sort by the pathologist under these circumstances is important because the number of donors and the volume of cross-matching may not be great enough to have given the medical technologists in the laboratory a wide experience in detecting unusual incompatabilities which may have potentially serious consequences if overlooked.

In the case of large blood banks in hospitals and elsewhere, the number of donors and volume of work may be so great that the services of one or more qualified physicians would be required full time for this purpose alone. It is almost impossible to induce competent physicians in sufficient numbers to engage in full-time work of such a restricted nature. It is not realistic to expect them to do so following a prolonged period of intensive training. Furthermore, the supply of qualified physicians is inadequate for such coverage. As a matter of fact, the competent medical technologist, because of the volume of work in such institutions, is often so skilled as to be entirely qualified for the task, provided that he or she can be relied upon to refer all problems of typing and cross-matching which cannot be resolved by routine methods to the physician for his professional judgment. It must be emphasized that it is the physician who must assume the responsibility for the release of blood from the bank in doubtful instances, whether the bank is small or large. I feel strongly, however, that as the extent and complexity of blood banking become greater, more and more responsibility must be placed in qualified and conscientious medical technologists. This is a policy which in practice should eventually have not only a strong moral but also a strong legal foundation.

The moral obligations of blood bank personnel are obvious to all of us. I think that the blood donor, potential and actual, has also a moral obligation which is not widely appreciated. It has been our experience that many a donor who presents himself at the blood bank is apt to belittle his past and present illnesses. He may even falsify his medical history in his desire to be an acceptable blood donor. Several instances have come to our attention in which blood donors suspected of being carriers of viral hepatitis concealed a previous attack of hepatitis when questioned at the time of blood donation. Concealment, furthermore, appears to be almost universal in the case of venereal disease. If he is a professional donor, he may be motivated to conceal because of his need for the fee. If he is replacing blood for a relative, his motivation in concealing his illnesses may be humanitarian. This observation applies to large blood centers, and it may well be that in smaller communities blood donors are more forthright in giving reliable medical histories. The hazards of the unreliable donor to the potential recipient in my opinion should be given wide publicity and personnel in donor stations should be made fully aware of the need to elicit the medical history meticulously. One can do little about the donor who is dishonest, but the uninformed donor who is honest in his belief that his previous illnesses are not now of consequence may be induced to reveal them to the examiner if skillfully questioned.

It is incumbent also upon the physician in charge of a patient to insure that he or his family is fully informed on his requirements for blood and of the dangers inherent in blood transfusion. I am afraid that the medical profession is not held in the high esteem which it once enjoyed. It is my impression that physicians today are often so preoccupied with the scientific and technical aspects of disease that they do not take the time to communicate adequately with their patients. An enlightened patient is more likely to be understanding and fair-minded

when an untoward incident occurs.

The patient himself or his next of kin in the case of incapacitated persons also have responsibilities toward transfusions and the blood bank. Agreement is general that persons have an obligation to replace blood which is used in their treatment. In my opinion, the patient has other obligations when he is hospitalized. I feel that he should realize that no therapeutic procedure is entirely devoid of risk even in the most competent hands. In spite of all reasonable controls in the operation of a blood bank, personal errors cannot be prevented entirely. When serious injury to the patient results, it is often a matter of moral and legal nicety whether the person or persons involved are justifiably liable for damages. Although I have not made a study of recent legal decisions against blood bank personnel, I gather from various sources that patients today are more apt to sue for injuries allegedly sustained during an operative or therapeutic procedure than they were likely to do in the past. It is also my understanding that proportionately greater sums of money are being awarded to plaintiffs for alleged injuries than in the past. To me this trend is very disturbing. Even though the patient is receiving ever better medical care, the implication is inescapable that some elements of the public either are losing confidence in the reliability of technical and professional medical personnel or are exaggerating their disabilities for their own gain. If this trend is allowed to continue, the risks may become so great that competent and reliable persons will be discouraged from engaging in blood banking. In the long run the best interests of the patient will not be preserved if this vicious cycle of events should develop. On the other hand, I believe that neither the courts or the public would permit complete immunity of blood bank personnel from liability for professional and technical negligence. Such immunity, in my opinion, would not be moral since one of the incentives to reliability would be removed. We, the Public, are therefore faced with a dilemma. Blood bank personnel should be held responsible for negligence and gross professional inadequacies. The Public however should exercise great restraint in suing for damages when negligence is uncertain or when damages resulting from human error are not permanent or lasting.

The legal profession should also be impressed with its responsibilities to exercise restraint on attorneys. Clients should not be encouraged to undertake litigation of dubious ethical and moral character, particularly on a contingent fee basis. Lawyers also need information on the difficulties in blood banking and the nature of transfusion reactions which cannot be entirely eliminated either because methods to do so are not yet available or because blood of identical group and type is not available in practical quantities. The legal profession thus has the obligation not only to serve the legitimate interests of its clients, but also to restrain its members from encouraging unprincipled litigation.

Conclusion

In conclusion, we are faced with two problems. The first is concerned with supply of blood itself. Some means must be found to induce more persons to donate blood on a voluntary basis. A nation-wide blood in-

surance program appears to be the best means. If voluntary programs are not successful, the Public will doubtless demand that insurance be undertaken by the Federal Government. In addition, the distribution of blood must be greatly improved. Again, it is virtually certain that if a voluntary organization does not succeed the pressure of public opinion will force the Federal Government to assume responsibility for a national blood program. The Public by its actions and support will eventually choose between the alternatives.

The second problem is one of supply of professional and technical personnel for blood banks. Not only is the demand increasing but the burden of moral and legal responsibility is becoming ever greater. If litigation for real and alleged damages becomes more common, competent persons may be discouraged from engaging in blood banking with the result that the supply of personnel may be curtailed and the quality of professional and technical competence may be dangerously lowered. We as blood banking personnel must strive for even better methods of safeguarding the donor and the recipient. Patients and lawyers in turn must exercise intelligent and unselfish restraint in instigating suit for alleged damages. Only by reliability, cooperation, and understanding among all parties concerned will the standards of blood banking be elevated and will the supply of blood and of blood bank personnel become adequate to meet the ever increasing demands.

THE DETERMINATION OF TOTAL AMINO ACID NITROGEN IN LEUKOCYTES*

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As long ago as 1922, Okada and Hayashi[9] made the observation, while working with leukemic bloods, that blood amino acid depended more on the number of WBC's than RBC's; they also found the concentration of amino acid nitrogen in the leukocytes to be 6 or 7 times the level in the blood plasma. Not much was done, however, in establishing the amino acid levels in the cells. During the past two decades, interest in the chemical composition of the leukocytes has been stimulated by the development of better micro methods; and the study of amino acids was given a great impetus when Martin and Synge[3] announced their new form of chromatogram employing two liquid phases. Such two-phase chromatography permits the separation of the amino acids found in a group, and the study of the identity and quantity of the individual acids present. The quantitation of the total amino acid levels may be considered a preliminary step in such a study.

The earlier methods which were used for measuring alpha amino acids were not very specific, but they had considerable usefulness. The earliest of these, Sörensen's formol titration was adopted for use with blood filtrates by Northrup[7]. The principle involved in this method is that, although it is not possible to titrate carboxyl groups of amino acids with NaOH directly, the addition of formaldehyde first makes such titration possible. Formaldehyde combines with the amino groups and prevents their reaction with NaOH.

Another method for the determination of aliphatic amino N in amino acids, peptides and proteins is the reaction with nitrous acid, and the gasometric measurement of the released nitrogen[11]. In this reaction, one mole of nitrogen is produced for each mole of free amino nitrogen, according to the equation:

$$H_2NCHRCOOH + HONO \rightarrow HOCHRCOOH + H_2O + N_2$$

Peptide bonds do not react. The liberated nitrogen is measured volumetrically in VanSlyke's blood gas apparatus, or in the manometric apparatus of VanSlyke and Neill.

Folin introduced a colorimetric method in 1922, the basis of which is the color developed by amino acids in the presence of beta- naphthoquinone sulfonic acid and alkali. A modification of this method by Frame, Russell and Wilhelmi[1] is still sometimes used.

The most specific method for the determination of free amino acids is the ninhydrin-carbon dioxide method of Hamilton and VanSlyke.[2] This is a gasometric procedure: ninhydrin reacts with alpha amino acids and sets free carbon dioxide and ammonia, molecule for molecule. Either the carbon dioxide or the ammonia can be measured: more accurate results are obtained when the liberated carbon dioxide is measured manometrically in the VanSlyke-Neill apparatus. Values by this method are somewhat lower than with the nitrous acid or formol titration methods, because the formation of carbon dioxide requires both a free amino

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group and a free carboxyl group on the same alpha carbon atom.

It is the photometric ninhydrin reaction, however, that has become the most widely used for the assay of free amino acids. An adaptation of one of these methods, that of Troll and Cannan,[10] will be described in connection with the analysis of leukocytes.

Preparation of the Leukocyte Sample

In order to obtain a sufficient number of leukocytes for testing, the quantity of blood to be drawn from each subject was varied, depending upon the leukocyte count. If the count was normal, 30 ml of blood was needed. If the patient was a leukemic with a very high WBC count, of over 60,000/cu mm, then 10 ml of blood was adequate to supply the required number of cells. From patients having a leukocytosis in the intermediate range, the amount of blood to be obtained was proportionally estimated. The blood was transferred from the syringe into a small Erlenmeyer flash containing 50 mg EDTA for each 10 ml of blood collected, and the flask was immediately refrigerated. It was transported as soon as possible to the laboratory in a small ice water bath. It is essential that the anticoagulant be EDTA, because by its chelating action, metallic ions which would interfere with the ninhydrin color development are removed from the blood[4].

The chilled blood was drawn up into long, siliconized glass tubing, having a length of about 60 cm, and a diameter of about 0.5 cm. One end was closed with a rubber stopper, and the tube placed in a refrigerator in a slanting position for sedimentation. The angle at which the red cells settled most rapidly was found to be an angle of 60° from the horizontal. Some investigators have added materials[5] such as dextran or fibrinogen to facilitate rouleau formation, but in general we did not consider this necessary. In a few instances, difficulty was experienced with the formation of cryoglobulins; when this occurred, sedimentation was permitted to proceed at room temperature. After standing in a slanted position for one hour, or longer if necessary, the tubes were changed to a vertical position for ten minutes. Then a long capillary tube was used to remove the supernatant plasma containing the leukocytes, and this was placed in a small vial. This sample of plasma and leukocytes was kept under refrigeration at all times when not in actual use.

The erythrocyte content of the plasma is reduced to a minimum by this technique; even with normal blood the RBC : WBC ratio is reduced to 2:1 or less without additives. The amino acid content of the erythrocytes is very low; and, at most, the error introduced by the contribution from the RBC is 5% of the total.

Duplicate WBC counts were performed on the well mixed plasma samples. Then a definite amount of plasma, calculated to contain 20-30 million leukocytes, was pipetted into each of several labeled lustroid centrifuge tubes. The tubes were centrifuged in the refrigerated centrifuge at 5°C for thirty minutes, using the high speed head at 13,800 rpm. With a medicine dropper having a fine capillary tip, the plasma was removed as completely as possible, and saved. The cellular sediment was

covered with parafilm, and immediately frozen. The total number of leukocytes in each tube was calculated from the quantity of plasma pipetted, and the cell count.

Lysis of the Leukocytes, and the Preparation of the Amino Acid Extracts

The cell samples were kept in the deep freeze for at least one-half hour. When desired, they were removed and thawed. Three small glass beads were added to each frozen sample. As soon as thawing was complete, (in two or three minutes), 0.1 ml of distilled water was added and the tube vigorously shaken. An additional 0.4 ml of water was added and the tube again shaken by hand until the mass was uniformly suspended. This was followed by twenty minutes of shaking in an adapted pipette shaker kept refrigerated at 5°C.

The proteins were precipitated by adding 0.5 ml of 10% trichloracetic acid drop by drop with shaking. The contents of the tubes were well mixed by lateral hand shaking, and then centrifuged at high speed for ten minutes at 5°C. Fritted glass Buchner funnels, which had been cleaned in dichromate cleaning solution and thoroughly rinsed, were placed in labeled, graduated centrifuge tubes. The supernatant from the trichloracetic acid precipitation was decanted into the appropriate Buchner funnel; the precipitate was broken up with about one milliliter of 5% trichloracetic acid, and recentrifuged. The supernatant and washings were drawn through the funnel by applying suction, and the filters were washed with an additional 0.5 ml of 5% trichloracetic acid to ensure a quantitative transfer of the amino acids in solution. The trichloracetic acid has a tendency to foam under reduced pressure; careful regulation of the suction is therefore necessary. The combined filtrate and washings should have a total volume of about 2.5 ml, and a record should be kept of this volume.

Since trichloracetic acid inhibits the ninhydrin color reaction, it was removed by shaking the TCA filtrate with two 30 ml portions of anhydrous ether[4]. A similar volume of 5% TCA was extracted to serve as a blank; and the leucine standard, diluted with an equal volume of TCA, was likewise treated. The pH was checked with p-Hydrion paper. Troll and Cannan claim satisfactory results with their method for any pH range of the aqueous solution between 1 and 8[10]. Extracts from the protein free cells usually had a pH of 3.5 to 3.6. If the pH is less than this, it may be regarded as evidence that the trichloracetic acid is incompletely extracted; and that, therefore, the specimen is unsatisfactory for chlorimetric analysis.

If necessary, the procedure may be interrupted at this point. If it is, the amino acid extracts must be frozen immediately. The amino acid level falls rapidly in small portions of solution, even when refrigerated at 5°C.

Selection of the Amino Acid Standard

In certain methods for amino acid determination, such as Moore and Stein's photometric ninhydrin method[6], the color yield from different amino acids, though reproducible, is not constant on a molar basis. With

the improvements introduced by Troll and Cannan, the color yield for sixteen amino acids is nearly 100% of the theoretical. The exceptions are the imino acids, proline and hydroxyproline, which produce a yellow color instead of the blue color of DYDA; tryptophane, which gives a 75% yield of DYDA; and lysine, which produces a 110% yield. All other amino acids tested give 98-102.6% of the theoretical color yield: that produced by leucine being 100% [10]. Leucine is, therefore, entirely satisfactory as a standard, although certain other amino acids might also be used.

The Ninhydrin Color Development, by the method of Troll and Cannan[10]

REAGENTS

1. Leucine standard solution: 13.1 mg leucine are dissolved in distilled water and made up to 100 ml. Each milliliter contains one microequivalent of amino acid nitrogen. This solution is distributed into small portions and kept frozen until needed.

2. 80% phenol solution: 80 grams of reagent grade phenol are dissolved in 20 ml of absolute alcohol, avoiding contact with metal such as spatulas, etc. Gentle heating may facilitate solution. This solution is shaken with about one gram of permutit for twenty minutes, then filtered. It is satisfactory for two weeks.

3. KCN-pyridine reagent: A 0.01 M solution of KCN is prepared by dissolving 65 mg of KCN in 100 ml of distilled water. 2 ml of this solution are placed in a 100 ml volumetric flask, and pyridine is added to mark. This solution is also shaken with about one gram of permutit for twenty minutes and filtered; it is stable for about two weeks.

4. Ninhydrin solution: 500 mg ninhydrin are dissolved in 10 ml of absolute alcohol, and stored in the dark. Fresh solutions should be prepared after three days.

5. 60% alcohol (v/V)

PROCEDURE:

Into each of two tubes graduated at 10 ml are pipetted duplicate 0.5 ml aliquots of the unknown aqueous solutions which should contain 1 to 8 gamma of amino acid N. Into a similar tube is pipetted 0.5 ml of extraced TCA to serve as reagent blank. Standard leucine solution is pipetted into additional tubes; 0.3 ml, 0.4 ml and 0.5 ml with enough of the extracted TCA to make the volume up to 0.5 ml. The standards thus prepared contain 0.15, 0.2 and 0.25 microequivalents of amino acid N respectively.

1 ml of 80% phenol in alcohol and 1 ml of the KCN-pyridine reagent are added to each tube, using a propipette. The tubes are immersed in a boiling water bath until they reach the temperature of the boiling water, approximately three minutes. 0.2 ml of the ninhydrin solution is added to each at timed intervals, the tubes are well shaken, stoppered with rubber stoppers, and returned to the water bath for exactly five minutes. The tubes are rapidly cooled in an ice-water bath; then 60% ethanol is added to the 10 ml mark, and the tubes mixed by inversion. The color of the solution after dilution is stable for at least one hour. The optical density is read on a Lumetron photometer at wave length 580 millimicrons. Values for the duplicate determinations are averaged, the amino acid nitrogen calculated and expressed in terms of micro-

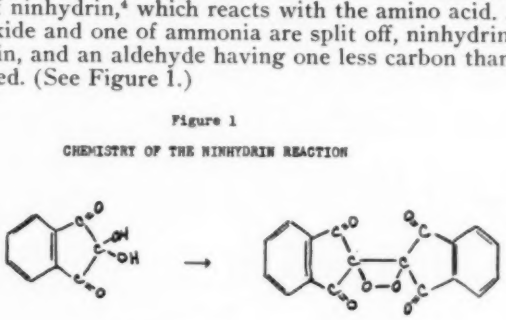
equivalents per 10^8 leukocytes.

Chemistry Of The Ninhydrin Reaction

In aqueous and in alkaline solutions, ninhydrin has an inactive form, triketohydrindene hydrate. Organic solvents, such as ethanol, favor the active form of ninhydrin,[4] which reacts with the amino acid. A molecule of carbon dioxide and one of ammonia are split off, ninhydrin is reduced to hydrindantin, and an aldehyde having one less carbon than the amino acid is produced. (See Figure 1.)

Figure 1

CHEMISTRY OF THE NINHYDRIN REACTION


triketohydrindene hydrate
active ninhydrin *
Active ninhydrin + $H_2NCHRCOOH \rightarrow NH_3 + CO_2 + RCHO +$
hydrindantin *
Hydrindantin + ninhydrin + $NH_3 \rightarrow$
Blue pigment, DYDA

* The structural formulae of the active forms of ninhydrin and its reduction product, hydrindantin, are according to Meyer (4).

The blue pigment, diketohydrindylidenediketohydrindamine or DYDA, is formed by the condensation of ninhydrin with ammonia and hydrindantin. Carbon dioxide does not form with proteins or polypeptides. For its production a free amino group and a free carboxyl group must both be present on the same alpha carbon atom.

Normal and Abnormal Levels of Amino Acids in Leukocytes

The bloods which were used for the determination of normal levels of leukocyte amino acids were obtained from male blood donors and from members of the laboratory staff, both male and female. Cell counts were done on the plasma which was used for analysis. As a result of

seventeen determinations, an average value of 8.05 microequivalents of amino acid N per 10^8 leukocytes was obtained, with a range of 6.7 to 9.8 microequivalents. One individual included in this series showed a change from 9.8 to 6.2 microequivalents per 10^8 cells after one week of a low calorie diet.

The findings of Nour-Eldin and Wilkinson[8] were somewhat lower than these results. They did 12 determinations on six normal subjects, and obtained an average value of 2.1 $\pm$ 0.1 microequivalents per 10^8 leukocytes. Their method of obtaining leukocytes was that of Minor and Burnett,[5] with sedimentation at 37° C; and for the measurement of amino acid N, they used the beta-naphthoquinone method of Frame, Russell and Wilhelmi.[1]

The bloods characterized as abnormal were obtained from hospital patients with a variety of conditions, most of which were accompanied by a leukocytosis. The cell counts as tabulated were done on the plasma which was separated after sedimentation; they should be a reliable index to the white blood cell count of capillary blood.[5] Plasma amino acid nitrogen levels which were obtained simultaneously are also included in the table below.

AMINO ACID N CONTENT OF PLASMA AND LEUKOCYTES FROM ABNORMAL BLOODS

Patient	Diagnosis	Date	Plasma WBC Count	mEq aaN/ml Plasma	mEq aaN/10^8 Leukocytes
RR	staphylococcus pneumonia	5/5	22,000	2.1	5.3
		5/7	35,200	1.9	4.2
		5/12	30,600	2.1	2.8
		5/18	47,000	2.5	2.8
		5/20	30,000	2.1	4.2
		5/27	12,600	2.2	4.5
	hip abcess	6/1	23,100	2.1	3.1
		6/3	25,000	2.1	3.3
EP	leukemoid reaction	4/23	43,900	2.6	5.5
		5/5	21,600	2.6	8.8
		5/7	15,300	2.7	11.7
		5/12	12,700	2.8	15.1
		5/14	18,100	2.7	13.8
MG	urinary infection	5/8	17,900	2.5	4.2
		5/11	18,000	2.6	4.0
		5/19	22,200	2.5	3.8
		5/26	14,900	2.7	4.4
		5/29	15,200	2.4	4.0
		6/2	13,000	2.8	3.6
		6/9	9,000	2.3	5.1
AW	Weil's disease	4/28	53,100	6.4	3.6
		4/29	deceased	...	...
WI	gastrectomy	4/21	15,000	3.0	7.8
		4/23	25,700	3.2	6.1
		4/28	7,100	3.1	5.6

The data presented here is insufficient for conclusions, but it does show that lowered levels of amino acid nitrogen may occur in the leukocytes when there is a marked increase in the number of cells. Further investigation would be needed to show the mechanism involved; that is, whether the decrease is due to competition for available amino acids, to defective uptake of amino acids from the plasma, or to increased

turnover by metabolically more active cells. This study was undertaken in conjunction with a study of amino acid metabolism of leukemic cells which will be reported separately. Our results suggest that the lowered levels of free amino acids in leukocytes reported in leukemia,[8] which we have confirmed, must be considered in relation to lowered levels found in other conditions in which there is leukocytosis.

Summary

A method is outlined for measuring the amino acid content of leukocytes; and values obtained from normal bloods, and a series of abnormal bloods, is presented.

Acknowledgment

The author wishes to express sincere thanks to Dr. Joseph B. Kopp, Director of Clinical Laboratories, E. J. Meyer Memorial Hospital, under whose direction this work was done, and to Allison Jefford and Henry Goller for much of the technical work.

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ANNOUNCEMENT

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RESEARCH OPPORTUNITIES ARISING IN THE DIAGNOSTIC LABORATORY*

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The word research has different meanings for different people. For writers of dictionaries, it means a laborious and diligent search after the true facts or principles. For people who sell soap or cigarettes, it means finding some desirable attribute that one's product possesses and that one's competitor's product lacks. For an army general, research may mean a chore that must be completed in order to send a rocket higher than an admiral can send one. For all of these people, and many more, research usually is associated with monotony, dullness, plodding —something that must be done, but, thank God, there is someone else willing to do it.

In contrast, most of the people engaged in doing research find their jobs to be fairly enjoyable and many of them actually look forward to Monday morning rather than to Friday afternoon. To be sure, if the sole objective of one's job is that of doing research, one will sometimes become stale and will look forward to and actually need a vacation. But the great majority of persons in this room, including myself, are not hired to do research and therefore, part-time research for us can add excitement and enjoyment to our working hours.

Practicing medical technologists are in a wonderful position, a position equalled only by clinical pathologists, to do part-time biomedical research. For one thing, you people have a constant flow of clinical material into your offices and laboratories; in fact, you can't stop this flow even if you wanted to. And this material comprises the most miraculous stuff in the universe. It includes not only the chemicals synthesized and fractionated by living matter—the hormones, the nutrilites, the metabolites, the antimetabolites, the toxins, the vitamins, and the antibodies—but even more fascinating—this material includes the living protoplasm itself. The clinical laboratory without a doubt contains the most exciting collection of substances ever assembled in one spot!

Secondly, you people are actually hired and paid to study this material. You are not asked merely to store the stuff, or to file it away as though you were a curator in a museum, but rather to observe, to evaluate, to understand these miraculous materials. And you are actually being paid to do this! As you well know, your studies of these materials can contribute significantly to the clinician's understanding of nutritional, the physiological, the pathological, and even the psychological status of the patient who supplied the material.

However you may not be quite so familiar with the possibilities of observing these clinical materials for features not previously recognized —features that may uncover new laws and principles in biology and medicine—discoveries that may lead to new cures or methods of prevention of disease—discoveries that may be of tremendous benefit to mankind.

* Address presented May 18, 1958, at the State Convention of the Ind. So. of Med. Tech. and Lab. Tech., Inc. Reprinted with permission from THE FUNNEL, the Indiana Society of Medical Technologists Journal and the Massachusetts Journal of Medical Technology. Received for publication August 1959.

For the remainder of this hour I will describe accidental and non-accidental discoveries that have been made in various areas of medical technology; discoveries that the laboratory personnel refused to shrug off as merely annoying or of no consequence but instead took the trouble to look into the cause of the phenomenon—in other words to do a little research. I hope to show you how simple it is to make observations and how simple it is to explore the observations—in fact, the only difficult things are to be sure to take a few minutes each day to do the research and to be sure to report it in a journal or at a meeting so that the fullest possible use can be made of your discovery.

Let's start with urinalysis. One of the most famous accidental discoveries made in connection with urine was that of the person who noticed that the urine collected from depancreatized dogs attracted an unusual number of flies. Just think for a moment of what you might do if you were the first to have made this observation. Among the more common reactions would be the following: "Let's get the janitor up here to spray the lab with fly-killer"; or, "Let's pour this urine down the sink before these flies drive us crazy"; or, "Let's ask for a transfer to serology." Or would your reaction be, "Now what ingredient is in that urine that makes it more attractive to flies than is urine from normal dogs?" Let's suppose you did ask yourself this question—would you then let the matter drop by saying to yourself, "Well, I'm not an analytical chemist so there's nothing further I can do." Or would you spend a few minutes each day, even though you are not expected or asked to do so, in examining the urine specimens in every way that you can? Would you spend an hour (perhaps even your lunch hour) in the library reading on what is known about the effect of the endocrine and non-endocrine glands on urine?

If your frame of mind is such that you are not annoyed by something unusual and if you are willing to think and to devote a little time to exploring an unusual occurrence, then you would have followed this last course. You would thus be in an excellent position to discover that the sugar content of urine of depancreatized dogs is unusually high. A little research project that you could easily do even in a small diagnostic laboratory would then reveal that the sugar content of the serum of these dogs is also abnormally high. And finally your publishing of the results of this project even in a brief note or abstract would ultimately lead to the discovery by endocrinologists of a chemical produced by the pancreas that could be used to treat diabetic persons, and this is how insulin was discovered. A point I should like to emphasize is that good research is simple research—in fact it is so simple that a common reaction of other people will be: "Why I knew that the pancreas was involved in diabetes all the time;" or, "Why, that's perfectly obvious." Of course the hairpin and the paper clip are simple and obvious objects but someone had to discover them and to tell other people about the discovery before they could be used.

Let's leave urinalysis and proceed to hematology. Have you ever stopped to think how many millions of differential smears have been examined by how many thousands of technologists before the discovery was made that approximately 1 in every 100 neutrophils possesses a drumstick projection on one of the nuclear lobes if the blood had been

obtained from a female instead of a male patient? I'm certain that the projecting structure on the nucleus must have been noticed quite often in the past but that attempts were not made to associate it or correlate it with the contributor of the drop of blood. How many other significant observations are we missing because we are merely asked to count the various types of white blood cells and we do just that and no more? I'm sure there are many persons, including myself, who have often seen unusual cells or bodies of some sort in this amazing material called blood but there are very few of us who have taken the trouble to think about these unusual forms and fewer still who have done a little bit of library and experimental research on the subject in an attempt to correlate the observation with the status of the patient. And yet we are all, as registered technologists, qualified by education and experience to do this. And in fact we have more opportunities to observe unusual forms in a blood smear than does a clinical pathologist or even a professional hematologist who would usually be observing smears only from patients suspected of having blood dyscrasias. In other words, a professional hematologist would rarely examine a blood smear of a patient admitted with, let's say, a brain tumor, yet the medical technologist would always have this opportunity.

Let's proceed to serology. Suppose you had stored a test tube of citrated blood in the refrigerator and the next day found that the erythrocytes were clumped. Would you merely shake the tube vigorously to break the clump and then proceed to use the blood sample without any more thought concerning this unusual occurrence, or would you do a little library and experimental research on the matter? If you were to follow the latter course you would need to spend only an hour or two in the library to learn if this observation had been published. If there were no articles on this phenomenon, you could easily institute a modest research project that would require very little of your time to determine how widespread the phonomenon of cold agglutination is and whether the possession of cold agglutinins in the serum could be correlated with a specific disease or a group of diseases.

You are all familiar with accidental discoveries made in bacteriology. Undoubtedly the most famous of all was that made in 1928 by Alexander Fleming. Just think how many petri plates contaminated with spores of penicillium mold have been discarded by bacteriologists and medical technologists before and since Fleming's discovery of penicillin. You may recall that Fleming was not looking for antibiotics at all but was merely observing colonial variation in plates containing staphylococci that were being allowed to age. Some day when you are in a medical library that subscribes to the British Journal of Experimental Pathology I would like you to take a few minutes to read Fleming's paper on penicillin which was published in 1929. You will see that every procedure carried out by Fleming could be done by each of us in a small and very modestly equipped clinical laboratory. And yet the paper gives a complete account of the nature and properties of penicillin and in fact equals the efforts of the modern drug companies who discover a new antibiotic and turn loose dozens of scientists and acres of laboratories to study their new antibiotic. I am also quite certain that Fleming, who graduated in 1903, had a much poorer education than any person in this

room yet he possessed something very precious—an attitude, a frame of mind if you wish, that allowed him to perceive more than just the results of the project with which he was routinely occupied.

Of course not all discoveries and research projects are initiated by accident. There are many problems that can be undertaken in a small diagnostic laboratory with a small amount of time each day that will lead to improvements in methods—and actually we, as medical technologists, have a responsibility to undertake these research projects ourselves whenever we are dissatisfied with a method. For example, some of you undoubtedly attended the ASMT convention in Chicago last year and perhaps heard Miss Isabel Havens of Billings Hospital present a paper on the use of agar in 4-ounce medicine bottles, rather than in petri plates, for solid medium blood cultures. Miss Havens and her colleagues had long wanted to culture blood in agar without the danger of petri plate contamination and finally decided to do something about it. They devised a bottle method, compared the bottle and petri plate method for 477 cultures, and obtained contamination only one-tenth as frequently in the bottle method. They found several other advantages inherent in the bottle over the petri plate method. Their paper was published in the ASMT journal last fall and perhaps some of you have already begun using agar media in bottles rather than in petri plates.

Let us next consider an example of a possible research problem in chemistry. We have all been trained to use as anticoagulants (when we need a sample of plasma rather than serum) either citrate or oxalate. And also we have been told that the addition of these calcium binding agents may possibly interfere with the chemical or biological test that we propose to run. For example, in assaying for enzymes that clot plasma we will obtain false positive results if the material containing the enzyme has the ability to ferment the citrate. And you all recall that a substitute such as heparin is expensive, cannot be safely autoclaved, and has other disadvantages. Then suppose you were sufficiently annoyed with citrate and oxalate for one reason or another and wondered about finding a good substitute—what would you do? Would you say, "Well, someone should do something about this but I am not competent to do it." Or would you think for a few minutes about the problem—perhaps while you are walking or riding to work or while you are washing glassware—and would you then think that what is wanted is a much more effective reagent that would bind calcium—a reagent that would be required in only one-hundredth or one-thousandth the concentration of citrate or oxalate and which would not be fermented by enzymes in clinical materials. And then, if you worked in or near Indianapolis, as many of you do, would you go to see Dr. Welcher at the downtown campus of Indiana University? Dr. Welcher as some of you know is a leading authority in the world on organic reagents for metals and has, in fact, written a four volume encyclopedia on this one subject. And if you would have visited Dr. Welcher (or if you merely consulted his encyclopedia in any science library) then he could have given you a list of some half a dozen reagents that are extremely effective calcium binders at the pH of blood. And as you may know, some of these reagents have been used in analytical chemistry for many decades and also have many industrial applications. It would be a simple matter

then to obtain small free samples of each of these reagents from the chemical manufacturers and test them as possible anticoagulants on an extra milliliter of blood taken from a patient. And if you had done this a few years ago you would have been the first person to discover the great advantages in using versene as a substitute for citrate or oxalate. You may say, "Well, someone did discover this, so why worry about it?" But there are literally hundreds of similar discoveries that are still waiting to be discovered—and these will be found—not by clinicians or administrators or professors but by bench laboratory workers—in other words—by medical technologists, by clinical chemists, and by clinical biologists.

Let's turn to parasitology which abounds with examples of discoveries made in the diagnostic laboratory. One such discovery was made very recently right here in Indianapolis at Eli Lilly and the complete consequences of this discovery are yet to develop. You all know that the kidneys of the monkeys imported from India are checked very carefully for latent virus or other microbial infections. One day a completely unexpected microorganism in a monkey kidney was discovered—only one single organism—an amoeba. This amoeba could easily have been overlooked since it resembled a monocyte, but it was not overlooked. It has been found to belong to a group of amoeba that were hitherto considered to be harmless. However, the Lilly people have begun a small research project on this amoeba. To the amazement of everyone, they find that the amoeba is extremely pathogenic and lethal to mice when it is inhaled by the mice and travels thru their olfactory nerves to the brain. There is much more to this fascinating story but you should hear it directly from the director of Biological Research of the Eli Lilly Company, Dr. Clyde Culbertson, and perhaps at a subsequent meeting of this group he would enjoy telling you this story in person.

Let's go on to histology. Some of you may have used in recent years the tetrazolium dyes to demonstrate areas of intense metabolic activity within various benign or malignant cells and tissues. But are you aware that these dyes had been discovered several years before they were first used in histology? Their first use was in agriculture where they are extensively employed to reveal the percentage of viable versus nonviable seeds. There is no good reason for the time lag between the adoption of these chemicals by agriculturists and by histologists. Most likely, the lag resulted from the fact that persons in histology do not read publications in chemical and agricultural research. And you can't excuse this failure to read because of lack of time for reading since each of the biological and biomedical professions publishes excellent condensed review articles on all phases of their current activities. I hope that at the very least, each of you reads the annual issue of: Methods in Medical Research, the Annual Review of Medicine, the Annual Review of Physiology, the Annual Review of Biochemistry, the Annual Review of Microbiology, Advances in Virus Research, and last but not least, Advances in Cancer Research.

I would like to conclude this morning with an example of research from an area of biomedicine that concerns all of us, not only as biomedical technologists but also as inhabitants of the world in the atomic age. The area I refer to, of course, is that of radiobiology. By far the

most important problem in radiobiology and in all of biomedical research is the discovery of a drug that would protect living cells from ionizing radiation.

As some of you know such a drug has been discovered. Oddly enough it was not discovered by radiobiologists in either the U. S. or in Russia although both countries have expended much effort in this direction. Rather it was discovered, in 1951, by a biologist in Belgium. Perhaps the Belgian's frame of mind is more seriously attuned toward defense against radiation than is ours or Russia's, because the Belgians have no means of offensive retaliation in atomic warfare. At any rate, the discovery of this drug provides a perfect example of the way to undertake rational research. The reasoning of the discoverer proceeded as follows: like all of us in this room he knew that there exist metabolites and antimetabolites—he knew that, for example, para-amino benzoic acid antagonizes the sulfa drugs; and that there are antagonisms among closely related molecules within the hormones, the vitamins, the amino acids, and so forth. Why, he reasoned, could not an antagonizing chemical be found for radiation damage? But, you may say, radiation damage is due to a physical rather than a chemical substance and you might give up this idea immediately. However, in 1943, the first of the chemicals that imitate the physical materials in radiation toxicity was discovered and since then several additional radiomimetic (or radio-imitative) compounds have been found. Each of you is familiar with the most well known of the radiomimetic compounds—it is used to inhibit rapidly growing cells just as is ionizing radiation—nitrogen mustard. Mustards in use at present include Mustargen and Leukeran. The chemical structure of the nitrogen mustards is known, and the Belgian radiobiologist reasoned that if he tested analogues or antimetabolites of this compound that he might find a drug that would prevent not only toxicity due to nitrogen mustard but also toxicity due to ionizing radiation. And he did —the drug is called cysteamine and our biologists at Oak Ridge have modified it slightly so that today it can be used in patients receiving mustards or X-ray therapy for cancer to prevent their normal cells from being damaged. Even more important, it is possible to take the drug by mouth about 20 minutes before entering a mildly radioactive area and one is then protected from coming down with serious radiation sickness. Unfortunately the drug is not concentrated in the gonads so that it offers no protection against the occurrence of undesirable mutations that might then be passed on to one's descendants.

Now I admit that it would be difficult for a lone medical technologist in a diagnostic laboratory to carry out a project involving the discovery of a drug that prevents radiation sickness. But I do insist that each of us could have carried out the thought processes that were involved in the discovery of cysteamine and could have then persuaded the radiologist and clinical pathologist of our respective hospitals to conduct a joint project of this type.

I think we can predict with certainty that most of us will eventually become involved with the drugs now available or those to be discovered that prevent sickness and death from radiation. Some of you in larger laboratories will be involved in research projects to evaluate the efficacy and the toxicity of these drugs. Many of you will perform diagnostic

procedures on patients who have received both radiation and these drugs. And sooner or later each of us will have these compounds in our medicine cabinets at home and perhaps will carry them at all times on our persons.

I should like to conclude by saying that I hope I have conveyed to you the following four thoughts. First, that part-time research is exciting and is fun. Second, good research is simple research. Third, many research projects arise from accidental discoveries made by persons with training like ourselves, but who have perceived more than merely meets an unthinking eye. And fourth, since we as medical technologists are the treasurers of the most miraculous stuff of the universe, we are actually obligated to do more with it than merely to assay it, to count it, to file it away or to pour it down the sink.

If each of you would conduct one part-time research project within the coming 12 months—no matter how large or how small a project—you would add to our modest collection of biomedical knowledge, you would add to the beauty and well-being of our precarious existence on earth, and you could take additional pride in being a member of a great profession—medical technology.

RELEASE

Diet and nutritional state are likely factors in radiation, according to an Air Force research study released to science and industry through the Office of Technical Services, Business and Defense Services Administration, U.S. Department of Commerce, Washington 25, D.C.

Tests on rats exposed to gamma radiation showed that effects of radiation varied with amount of protein content in the animals. Another study of the effect of radiation on organic materials also is available through OTS. The two reports are:

CHRONIC EFFECTS OF LOW-LEVEL RADIATION UPON PROTEIN AND AMINO ACID REQUIREMENTS. B. H. Ershoff and others, Western Biological Laboratories, for Aerospace Medical Laboratory, Wright Air Development Center, U.S. Air Force. Dec. 1959. 54 pages. (Order PB 161501 from OTS, U.S. Department of Commerce, Washington 25, D.C., $1.50.) Results of this study indicate that much of the effects of radiation are dependent upon an animal's nutritional state and diet. Immature male rats were fed prified diets containing various degrees of protein and exposed to gamma radiation for periods as long as 20 weeks. Control groups were fed the same diets but not irradiated. Alteration of protein content did not change radiation effects. Learning ability was affected by continuous exposure to gamma radiation. Bibliographies and data tables are included.

CALCULATION OF ABSORBED DOSE. C. H. Cheek and V. J. Linnenbom, Naval Research Laboratory, U.S. Navy. Feb. 1960. 34 pages. (Order PB 161259 from OTS, U.S. Department of Commerce, Washington 25, D.C., $1.) This report presents equations and tabular data for calculating the absorbed dose in rats when conditions of exposure to radiation are known. Organic substances such as plastics, elastomers, lubricants, and insulating materials contain elements to which this study was devoted. Effects investigated included gamma radiation, monoergic electron beams, and neutrons having a spectrum of energies.

ABSTRACTS

PURE RED-CELL ANAEMIA AND THYMOMA

A thymoma is a tumor derived from the epithelial elements of the thymus. The association of benign thymoma and red-cell aplasia is a rare striking combination, some 18 cases having been described in the literature. The picture is one severe anemia without any depression of WBC or platelets.

This article is a case report of a patient having 6.1 gms. % Hgh.; 2.3 M RBC; 11 vols. % Hct.; MCV 80 cu. μ, MCHC 28%; WBC 6,000 (Neutrophils 60%, Lymphocytes 30% and Monocytes 10%). There was marked hypochromia and marked polychromasia. Microcytosis and some anisocytosis were also seen in later films. The reticulocytes were below 1% on 4 occasions.

"Pure" red-cell hypoplasia is characterized by (1) a chronic, profound normocytic normochromic or macrocytic normochromic anemia with a decrease or absence of reticulocytes (2) a normal differential count and usually a normal total WBC, although leukopenia or a leukocytosis can occur (3) normal platelet levels and an absence of hemorrhagic phenomena. In general, the anemia is severe, the Hgb. averaging 4.2 gms. % in 8 cases, the lowest being 1.5%.

In this case there was no improvement in the blood picture after removal of the tumor up to the time of death 18 days later. Freeman, Z., Prince of Wales Hospital, Randwick, New South Wales, Australia. *British Medical Journal* May 7, 1960, pp. 1390-1392.

MEGALOBLASTIC ANAEMIA DUE TO PHENOBARBITONE

Megaloblastic anemia may develop in patients treated with the anticonvulsant drugs phenytoin sodium and primidone and in patients receiving large doses of barbiturates. Although a mild macrocytosis has been observed in about ⅓ of patients receiving phenobarbitone, megaloblastic anemia due to this very widely used barbiturate has not been recorded.

This paper describes the megaloblastic anemia occuring in one patient while taking large doses of phenobarbitone.

A blood count showed RBC 1.2 M; Hgb. 5.5 gms. %; Hct. 17 vols. %; Reticulocytes 0.1%; MCV 141 cu. μ; MCHC 32%; WBC 4,200 (Neutrophils 74%, Lymphocytes 16%, Monocytes 6% Eosinophils 4%), platelets 125,000. The stained film showed macrocytosis, poikilocytosis, anisocytosis, hypersegmentation of neutrophils, and occasional myelocytes. Chanarin, I., Laidlaw, J., Loughridge, L. W., and Mollin, D. L., Departments of Hematology and Medicine, Postgraduate Medical School of London, London, W. 12, England *British Medical Journal* April 9, 1960, pp. 1099-1102.

THE PART THAT CAN BE PLAYED BY THE COLLEGES IN BETTER QUALIFYING THEIR GRADUATES FOR CAREERS IN HEALTH LABORATORIES†

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Consideration of the part that can be played by the colleges in better qualifying their graduates for careers in health laboratories may be approached from three viewpoints:

(1) From that of the colleges; what is the purpose of a college education?

(2) From that of the future employer; what has the student been taught and been taught to do? and

(3) From that of the student; what courses are offered in the college of his choice and which ones will be of the greatest value in a career in health laboratories?

We will all agree that the primary purpose of a college education is to acquaint the student with the experiences of past generations and to develop in the student the ability to think for himself. Learned faculties have set up curricula for baccalaureate degrees for the purpose of giving the student a broad background upon which he can build the career of his choice and which will make him a better member of the human race. It would be presumptuous for us to think we should suggest courses and course content to the universities and colleges, especially the liberal arts colleges, which would resent anything suggestive of a "trade school."

The future employer of the student would like him to be instructed in subjects which will give him a good background for understanding the work he is to undertake. If that instruction can also give the student some know-how of performance the employer will have far less "on the job training" to do before the student begins to produce acceptable work.

The student has to decide on the college from which he will receive the education he wants. If the length of time he can spend in acquiring an education for the career of his choice is a critical factor he may be willing to settle for a "trade school" education. If he wishes to continue his education after acquiring a baccalaureate degree and wants a good foundation for his graduate work he will select a college rich in liberal arts and sciences. If he is really wise he may try to gain some knowledge about the members of the faculties of the colleges and to learn about their contributions to their fields which may be of special interest to the student and give him an insight into the type of work he might be doing after graduation.

Just what courses should a student take in college to prepare himself for a career in a health laboratory? What courses are found most commonly in the curricula for baccalaureate degrees? These are usually divided into group requirements and requirements for concentration in a major and minor subject. For the future health laboratory worker probably his major subject will be in one of the sciences, biology, chemistry

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or physics. The group requirements, in addition to furthering his knowledge of the literature of his native language will also acquaint him with one, preferably two, modern foreign languages, so that he will acquire facility in communication and in reading current publications on work in his field. Group requirements will also give him good foundation in mathematics which will be useful in courses in physics, chemistry and biology. Lastly, the group requirements through psychology and the social sciences will give him a knowledge of human beings and their behavior and what makes them do the things they do. The person with a liberal education must know how to get along well with his associates and to do this he must know that we all have our frailties, we all want some recognition and we all need some pampering of our "egos." If he is to assume leadership in his field and to direct the work of others or to help with the instruction of novices, he will need some knowledge of how to evaluate his fellow workers.

What science courses are of the greatest value to the future health laboratory worker? He must have basic knowledge of the work of his chosen field. Just what that work is to be may be difficult to ascertain at this period of his education. Should he prepare to work in one field or to be able to adjust to more than one field? If he is to be a sanitarian, he needs chemistry, microbiology and certain aspects of engineering. If he wants to confine his work to chemistry he needs courses through organic and at least elementary physical chemistry. These courses require college mathematics through elementary calculus. If he is to work in microbiology he still needs courses in chemistry and college mathematics because microbiology is the study of the life of microorganisms and all life processes are gradually being explained in the chemical terms used in the field of biochemistry. With the increase in instrumentation in laboratory work, he needs a knowledge of physics and even electronics to understand the mechanics of these instruments to use them to best advantage. If he is to work in the field of cancer research, and cancer is a public health problem, he needs a knowledge of chemistry, anatomy and histology to do the many aspects of histo-chemistry, tissue culture and virus work. He might even profit by a knowledge of pharmacology, if he were to participate in chemo-therapy studies.

What of the course content of these subjects? In the undergraduate colleges, the "ivy-towers," course content should be rich in theory and principles of procedures which might be used in the health laboratory. Laboratory exercises designed to illustrate these principles and the theory in back of them should be used to acquaint the student with the handling of apparatus and the tools used in the procedures. In some colleges, specific techniques which have been devised for practical application of these principles and theories for use in health laboratories can be taught the student. However, care must be taken to avoid limitation of the instruction to the acquiring of manual dexterity only, the student needs to know why he gets an unexpected, or erroneous, result. If it is an error, what went wrong and why. If it really is an unusual result, is it valid? He needs to know how to evaluate the results of a test, are they usable in a survey of health conditions, do they tell the whole story or is there some angle of the technique, some quantity of a reagent

which is responsible for the results obtained which differ from those of another technique used for the same purpose. Some of this knowledge cannot be taught in undergraduate courses nor even in graduate courses, but comes from actual experience in the laboratories. However, some of it can be given the student by experienced instructors.

If he is to go on to graduate study and into research work he needs a thorough grounding in scientific knowledge in the field of his choice so that he may have at his fingertips the knowledge needed to solve any problems he encounters. He needs to know how to ask intelligent questions about a subject and then to know how to go about finding the answers to these questions. Some of these problems will be given him by his employer and his value to his employer will depend on the ease with which he can solve these problems. Sometimes the problem will be narrowed down to how well and how quickly he can get through the work assigned him for the day, or week, or month, and it may be years before he is in the position to demonstrate his ability to work on a problem of his very own. When the time does come for his own independent research his ability to think for himself as acquired through his college education will play a large part in the successful progress of his work.

Just what can the universities and colleges do for the student who is preparing for a career in health laboratories? The liberal arts colleges can adapt their admissions policies to ensure enrollment of good students, those with good college entrance scores and the proper motivation for a career in health laboratories. The colleges can set up counselling programs which will guide and advise the student about his courses and their sequence during his entire undergraduate period of study. Colleges might confer with health laboratory staffs as to the subject matter to be emphasized in courses most useful to the laboratory worker. Representatives from the health laboratories might be invited to take part in seminar courses at the colleges.

One approach to this problem has been taken by a few of the universities where special undergraduate professional schools have been established which offer opportunities to students interested in careers in the health field other than medicine. The School of Allied Medical Professions at the University of Pennsylvania was started in 1950 when the Philadelphia School of Occupational Therapy and the School of Physical Therapy at Graduate Hospital were brought into the undergraduate program of the University. The Division of Medical Technology was added in 1952 and a degree program in Oral Hygiene in 1957. Students are admitted to the School through the Office of Admissions of the University as Freshmen and are under the advisory council of the Faculty of the School throughout their undergraduate life. The School has its own Faculty, Executive and Educational Committees. Group requirements are fulfilled by courses in the College of Liberal Arts and Sciences. Courses in the field of major specialization are given during the third and fourth years. These courses, while emphasizing general principles of the specialty, give some practical applications of these principles. However, the professional education of these students is not attained until a period of satisfactory clinical practice, which varies in length from four months for the Physical Therapists, to 10 months for the

Occupational Therapists and 12 months for the Medical Technologists, has been completed.

The program for the medical technologists should be of special interest to those students interested in health laboratory work. Group requirements in this program include courses in English, a foreign modern language, chemistry, through organic; courses in biology, which include general physiology, parasitology, hematology and microbiology; courses in the social sciences and a few electives. Major specialization begins in the third year when courses in diagnostic bacteriology, mycology, serology and virology, diagnostic chemistry and instrumentation, a course in principles of tissue structure, and medical technology orientation are given. The fourth year is spent in the laboratories of the Hospital of the University of Pennsylvania where the students rotate through the departments of the clinical laboratory. During this year daily lectures and/or seminars are given the students in one or the other of these departments. As part of their orientation in medical technology these students are taken to visit the Pennsylvania State Public Health Laboratories and, for the past four years, they have spent one month of their senior year in the Parasitology department there. In addition, the seniors take one semester course in a social science subject in the College of Liberal Arts and Sciences. When they have satisfactorily completed their 12 months of clinical laboratory practice they are given the degree of Bachelor of Science in Medical Technology from the University of Pennsylvania and are eligible to take the examination of the American Society of Clinical Pathologists for registration as Medical Technologists. Although all these courses in medical technology deal with techniques, they stress the principles of the tests and the science upon which these principles are based. None of them are "snap" courses and prospective students are warned about the difficulties of these courses.

To set up a program for the undergraduate college student which would include basic courses in all the sciences would result in a curriculum unacceptable to any liberal arts and sciences faculty interested in the attainment of a broad educational background for the student. There just would not be any time left for the cultural courses which make life more enjoyable for the student. The resulting program would be so difficult, far more difficult than that required of the pre-medical student, that only the exceptional student exclusively interested in and capable of understanding all branches of science would be willing to attempt it as an undergraduate. In many universities today there is a trend toward more liberal arts and courses in the humanities in the undergraduate programs and a de-emphasis of professional training for the undergraduate. Most of the larger universities lean toward postponement of all professional education until the student is ready for graduate school.

The student interested in a career in the health laboratories can find universities and colleges which will give him the basic science courses he needs for his chosen field. Educational councillors can give him guidance in selecting his college and in selecting the courses most useful to him. A few universities have established undergraduate professional

schools for people interested in careers in the fields of health allied to that of medicine.

The student who wishes to be a success in this field, the student who will be of greatest value to the staff of the health laboratories, must be motivated to service for his fellow man and must be made aware of the difficulties of the subject matter he must master to enable him to pursue a career in health laboratory work. He must be willing to work and to work hard to attain his goal. However, in order to interest a graduate who has received good undergraduate training in courses which will be useful to him in a career in the work of health laboratories, something should be done by the employer to make it worth the while of the student to pursue the difficult courses which will fit him for his career. He will want to be rewarded, not always just by money, although it helps, but by recognition for attainment and by opportunities for advancement in his career.

Finally, mention might be made of one other problem which confronts the universities with undergraduate schools of allied medical professions. That problem is the one of recruitment of good students. Knowledge of these undergraduate professional schools has not been made available to high school and college students in a manner to arouse their interest. Some high school and college counsellors either know little about such courses or dissuade good students from entering programs which would prepare them for careers in health laboratories by saying the opportunities for advancement in this field are limited.

Health Career Days sponsored by members of the Laboratory Section of the American Public Health Association or the Conference of State and Provincial Laboratory Directors or, possibly, arrangements for the showing of films and exhibits at a special session of one of these groups to which high school and college students and their counsellors could be invited might arouse the interest of good students and lead them to apply for admission to the universities which have schools of allied medical professions.

MEDICAL TECHNOLOGISTS WANTED

Two or Three Technologists registered MT (ASCP) and eligible for registration by the California State Department of Public Health. Starting salary $500.00 per month. Night calls for one week in every four or five. Technologist reimbursed at rate of $5.00 per call. Applications should be accompanied by a transcript of all college records, photograph, and recommendations by former employers. Write: Philip Flynn, M.D., Pathologist, 2005 Court Street, Redding, Calif.

Wanted: Registered ASCP Medical Technologists for hospital now building in Trenton, Michigan, a unit of Peoples' Community Hospital Authority and to have 80 beds initially. Salaries range up to $500 from a starting level of $410 per month. Starting salary depends on previous experience. Guaranteed annual increases. Apply to: Director of Laboratories, Outer Drive Hospital, 26400 Outer Drive, Lincoln Park, Michigan.

Medical Technologists—Wayne County General Hospital—Salary: $5537 per year (Effective 9-5-60 subject to approval of the Board of Supervisors). Present rate $5057 to $5537. Requirements: U. S. Citizenship; not past 41st birthday; A.S.C.P. registration or 3 years college and completion of 1 year training in an AMA approved laboratory. Contact Wayne County Civil Service Commission, 625 City-County Building, Detroit 26, Michigan.

Wanted: Registered Medical Technologist (ASCP) male or female to head department of blood banking and hematology in active, clinic affiliated hospital undergoing expansion to 240 beds. Beautiful location. Year round weather excellent. No night call. Starting salary $4,800 with regular increases. Contact Dr. D. N. Tweeddale, Pathologist, Trover Clinic, Madisonville, Kentucky.

Medical Technologists, ASCP registered or eligible for progressive teaching hospital. Laboratory departmentalized. Air conditioned and modern approved Medical Technology Training School (AMA). Forty hour week with paid vacation and sick leave. Salary commensurate with education and experience. Progressive salary increase program. No night calls. Interested parties contact: Mrs. Marilyn Baehr, MT (ASCP), Technical Supervisor, Mount Sinai Hospital, 2750 West 15th Place, Chicago 8, Illinois.

Wanted: Two A.S.C.P. Registered Medical Technologists; new 157 bed hospital fully accredited; modern laboratory fully equipped to do all lab procedures. Beginning salary $320 month with annual increments plus call pay. 40 hr. week with 2 weeks paid vacation, 12 days sick leave, and 6 paid holidays annually. Housing available for female employees. Group insurance and credit union membership available. Recreational facilities abundant. Famous Sun Valley only 2 hours distance by auto. Apply Raymond L. Tate, Asst. Admin., Magic Valley Memorial Hospital, Twin Falls, Idaho.

A SIMPLE METHOD FOR DETERMINING RED BLOOD CELL POTASSIUM*

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INTRODUCTION

Several articles[2, 4, 5] have reported the significance of changes in the concentration of the intracellular potassium in certain pathological diseases. From the work these investigators have done, it has been shown that a loss of potassium from the cells does not necessarily mean there is a reduction in the extracellular or plasma potassium. It has also been found that a low extracellular potassium concentration can occur without a reduction in the potassium of the cell.[6] From these findings it has become quite evident that we cannot predict the amount of potassium in the red blood cell or other body cells from a study of the plasma only. Evidence of these evaluations has also been shown in many electrocardiographic tracings.[3] In some instances there has occurred a flattening of the T wave which usually indicates a depletion of the body potassium. However, upon analyzing the plasma, the potassium was found to be normal, and sometimes even elevated. This could mean that this depletion might be occurring in the cardiac muscle and thereby being reflected in the red blood cell.

From the investigations that were done, and from the peculiar findings in the electrocardiograms, it became quite evident that a rapid, clinically applicable method for determining red blood cell potassium was needed. Although there are methods for determining red blood cell potassium, one of the problems has been the lack of a simple method or technic whereby the analysis could be carried out as a routine study in the clinical laboratory. The purpose of this report is to present a method which will make possible the determination of intracellular potassium as a routine procedure as the demand and need for this analysis increases.

METHOD

Reagents[1]

Stock lithium solution, 2.0 N
84.8 gm. LiCl are dissolved in distilled H_2O and diluted to one liter.

Dilute lithium solution
5 ml. of stock lithium diluted to 1 liter with distilled H_2O.

Stock mixed sodium and potassium standard
KCl (dry) 0.4475 gm. and NaCl (dry) 11.6908 gm. are dissolved in 1 liter of distilled H_2O. This solution contains 6 mEq. of potassium per liter.

Dilute mixed standard
1 ml. stock standard plus 100 ml. dilute lithium solution (1—101 dilution).

Aqueous heparin sodium
50 mg. per cc.

* Second Award, Biochemistry, Scientific Products Foundation Awards, June 1960. Read before the 28th Annual Convention of ASMT, Atlantic City, New Jersey, June 1960. Received for publication January 1960.

PROCEDURE

Collection

Collect 10 ml. of blood with minimal trauma in a syringe in which the walls have been moistened and the needle lumen filled with aqueous heparin. Avoid exposure of blood to air after collection by inserting the needle into a rubber stopper.

Before analysis, transfer the blood to a clean, dry test tube and stopper with a clean rubber stopper. Invert carefully, being sure not to shake, at least 8 times before each sampling.

Determine a hematocrit value for each specimen by any standard procedure. This will be used later in calculating the result. We have used both the Wintrobe and capillary methods.

Preparation for analysis of red blood cell potassium

Prepare a 1—11 dilution of whole blood by adding 1 ml. of whole blood to 10 ml. of distilled water. Mix well by inversion until the tube appears to be a clear red (all cells are then hemolyzed). 0.2 ml. of this dilution is then pipetted and rinsed from a "to contain" capillary pipet into 20 ml. of dilute lithium solution, (1—101 dilution).

Preparation for analysis of plasma potassium

The whole blood specimen is then centrifuged and 0.2 ml. of plasma is pipetted and rinsed into 20 ml. of the dilute lithium solution, (1—101 dilution).

Notes on technic

With each pipetting, the pipet is pre-rinsed with the solution to be transferred. This portion is discarded. The sample is then taken for transfer.

Homogeneity of the whole blood sample is essential when sampling for hematocrit and for whole blood potassium.

Analysis

Specimens and standard were read on a Baird Flame photometer with a setting of 150 for the standard.*

CALCULATION

Principle:

The concentration (mEq./liter) of potassium in the red blood cell may be calculated from the following equation.

$$C_{rbc} = C_{plasma} + \frac{C_{whole\ blood} - C_{plasma}}{Hct.}$$

where

Hct. = hematocrit fraction (50% hematocrit = 0.5)
C_{rbc} = mEq. potassium per liter of red blood cell mass
C_{plasma} = mEq. potassium per liter of plasma
C_{blood} = mEq. potassium per liter of whole blood

* This setting is arbitrary and depends on the flame photometer used and the concentration of dilute lithium.

DERIVATION OF FORMULA

$$C_{blood} = C_{plasma}\,(1\text{—Hct.}) + C_{rbc}\,(Hct.)$$

$$C_{rbc}(Hct.) = C_{blood} - C_{plasma}\,(1\text{—Hct.})$$

$$C_{rbc}(Hct.) = C_{blood} - C_{plasma} + C_{plasma}\,(Hct.)$$

$$C_{rbc} = \frac{C_{blood}}{Hct.} - \frac{C_{plasma}}{Hct.} + \frac{C_{plasma}\,(Hct.)}{Hct.}$$

$$C_{rbc} = C_{plasma} + \frac{C_{whole\ blood} - C_{plasma}}{Hct.}$$

EXAMPLE

Hematocrit value = 44% (hematocrit fraction 0.44)

	Readings	mEq./liter
whole blood potassium	106 (1:11 dil.)	47.7
plasma potassium	105	3.86
standard	151	

$$\text{mEq. of potassium/liter} = \frac{R_u}{R_s} \times 6\ (\times \text{preliminary dilution})$$

$$\text{potassium of the red blood cell} = 3.86 + \frac{47.7 - 3.86)}{0.44}$$

$$= 103.5\ \text{mEq./liter or } 103.46\ \text{mEq./liter}$$

RESULTS

TABLE I

Patient	mEq./liter	
1	90.2	
2	90.2	
3	87.2	
4	98.7	
5	99.3	
6	99.0	
7	101.0	mean = 96.2
8	103.6	
9	88.8	standard deviation = ± 5.1
10	94.9	
11	97.8	
12	98.1	
13	101.3	
14	105.5	
15	88.0	

Fifteen normal patients were studied and the average red blood cell potassium was calculated as 96.2 mEq./liter of red blood cells with a standard deviation of ± 5.1 (see Table I).

In patients in which the red blood cells were depleted of potassium, we obtained results as low as 70.0 mEq./liter.

DISCUSSION

We have selected two cases of potassium red blood cell depletion

which we feel are significant enough to demonstrate the need for this type of study in the clinical chemistry laboratory.

Case I

T. R., aged 66, white female, was admitted to the hospital with severe mid-abdominal colicky pain and loose brown diarrhea of 3 days duration. She was diagnosed as having mesenteric thrombosis. Twenty-five cm. of small bowel were resected and the patient was digitalized after showing signs of congestive heart failure. Soon, paroxysmal auricular tachycardia with block supervened and the patient became moribund. Her electrocardiogram revealed S T depression, T wave inversion and a prominent U wave. The serum potassium at that time was 3.9 mEq./liter and the red blood cell potassium decidedly abnormal, 72.1 mEq./liter. One week later the serum potassium also showed a decided decrease, 2.6 mEq./liter. Potassium chloride was administered for two weeks. The patient was given 4 gm./day and then increased to 8 gm./day. By the end of the two weeks she was receiving 16 gm./day. At that time the serum potassium was 4.0 mEq./liter, and the red blood cell potassium was 93.0 mEq./liter, indicating normal potassium electrolytes. The electrocardiogram showed no sign of T wave depression, again indicating normal potassium restoration.

Case II

L. L. W., aged 63, colored female was admitted with a diagnosis of nephrotic syndrome and treated with cortico-steroids. In spite of treatment the patient became progressively weaker with concurrent loss of reflexes. Her serum potassium was high, (see Table II) but the electrocardiogram showed a lowering of the T wave in the precordial leads indicating the possibility of a low red blood cell potassium. The intracellular study showed the potassium to 71.6 mEq./liter which is abnormally low, and potassium chloride was administered intravenously, (see Table II). Within a few days the red blood cell potassium rose progressively until it reached normal limits, at which time the electrocardiogram showed a normal tracing. It is interesting to note that the amount of potassium chloride administered was based on the result of the red blood cell potassium on the previous days and that the serum potassium was of no value in estimating potassium requirement.

TABLE II

Date	Serum Potassium mEq./liter	Red Blood Cell Potassium mEq./liter	Potassium Chloride Gms. given/day
8-3 -59	5.6		
8-5 -59	5.0		
8-7 -59	5.8		
8-10-59		85.1	1.2 gm.
8-11-59			4.0 gm. x 3 days
8-12-59	6.0		
8-14-59		71.6	8.0 gm. x 7 days
8-17-59	6.6	83.8	
8-18-59		97.5	
8-20-59	6.5	91.5	
8-24-59		90.7	
8-26-59		94.4	
8-30-59		95.7	
8-31-59	4.8		

We have established the normal values for red blood cell potassium by our method and have shown significant changes in the red blood cell potassium in some pathological disorders. Keitel and his associates have done a great deal of work on the problem of intracellular electrolyte changes.[4,5] We feel that his method of analysis in terms of millimoles per kilogram of solids is more accurate, and preferable (in theory) to the one here presented. However, Keitel's method requires several weighings and dryings which are time consuming, and necessitate a delay in results and treatment. We feel we are justified in sacrificing Keitel's accuracy in exchange for a rapid method which can be used with satisfactory results as a routine determination. A red blood cell potassium can be determined within one hour by any technologist familiar with the flame photometric technic.

SUMMARY

A rapid method for determining red blood cell potassium has been adapted for the Baird flame photometer.

The technic is simple, reliable, and lends itself readily to the clinical laboratory for routine analysis.

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6. Schribner, B. H., Freemont-Smith, K., and Burnell, J. H., The Effect of Acute Respiratory Acidosis on the Internal Equilibrium of Potassium, J. Clin. Invest. 34: 1276-1285, 1955.

MEDICAL TECHNOLOGISTS WANTED

Medical Technologists: ASCP registered or eligible, 503 bed general, non-profit, non-denominational hospital. Laboratory semi-departmentalized, air conditioned. Progressive department; quality controls; research; individual work encouraged. Approved M.T. Training School. Staff of 32, excl. students; 80% MT (ASCP). Starting salary new graduates $346 per month, merit increases to $423. 40 hour week; overtime pay. Two weeks paid vacation (first year); sick leave; legal holidays, sickness, accident, and group life insurance available. Write or call: R. W. Myers, Aultman Hospital Laboratory, Canton 10, Ohio.

Medical Technologist, ASCP registered or eligible. 40 hour week. General Duty. $360 per month. Contact: G. W. Thoma, St. Mary's Infirmary, Galveston, Texas.

Technologist: ASCP registered or eligible. General experience for clinical laboratory. Small Chronic Hospital. Maintenance for single person. Salary $4500-$4800. Retirement and other benefits. Write, Levindale, Baltimore 15, Maryland.

Medical Technologists: ASCP registered or eligible. (Individuals with equivalent experience & bachelor degree considered also.) For Hematology, Blood Bank, or Bacteriology departments of expanding 900-bed hospital located in Chicago's Medical Center. University of Illinois affiliation. Top salary for 40 hour week—5 days. Employee benefits include free individual Blue Cross Insurance after one year, sick leave accrual, three weeks vacation. Hospital owned apartments available at low rental. Send resume and photo to Personnel Department, Presbyterian-St. Luke's Hospital, 1753 West Congress Parkway, Chicago 12, Illinois.

Medical Technologist, ASCP or eligible. All hospital lab. work. Supervise 3 MT's. 160 bed hospital, 40 miles north Columbus, Ohio. $400 mo., advancement. Some call time. Immediate appt. Beautiful community of 17,000. Contact Personnel, Mercy Hospital, Mt. Vernon, Ohio.